Office of Naval Research
Annual Productivity Report 2002-2003

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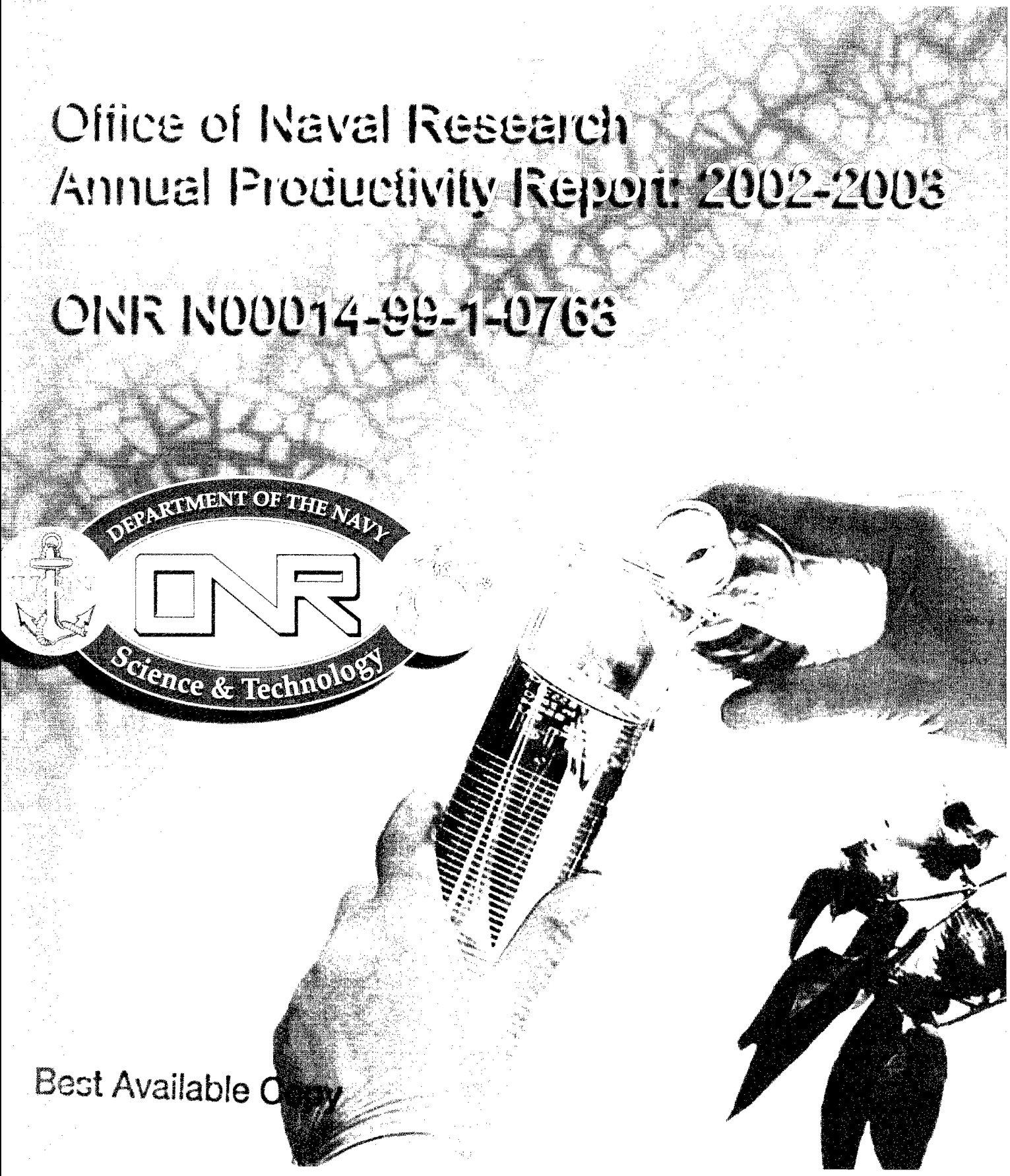
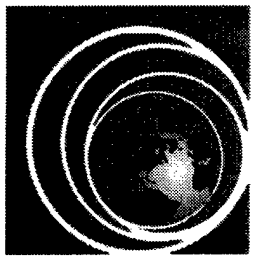
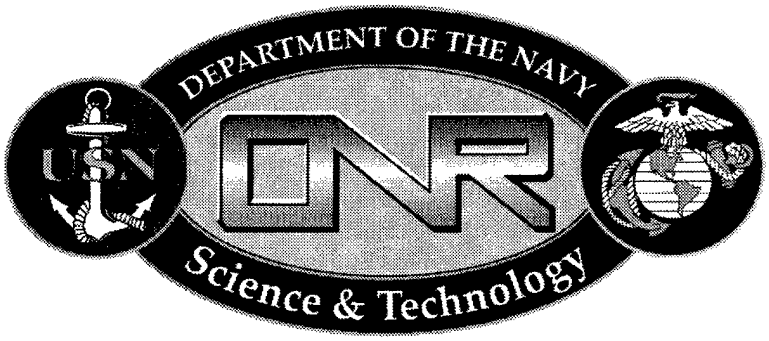




CENTER FOR
BIOENVIRONMENTAL RESEARCH
at Tulane and Xavier Universities

Integrated Bioenvironmental Hazards Research Program

US Department of the Navy
Office of Naval Research



Productivity Report, 2002 - 2003
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INTEGRATED BIOENVIRONMENTAL HAZARDS RESEARCH PROGRAM

ANNUAL PRODUCTIVITY REPORT

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Center for Bioenvironmental Research At Tulane and Xavier Universities
Bioenvironmental Hazards Research Program
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ABSTRACT

Beginning in April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. The BHRP program includes mechanisms for the effective communication of this information for resolution of Department of Defense problems and for the educational training of future scientists.

One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health; and development of biosensors for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or extending to ecological and ecosystem function levels. Given the CBR's and Navy's mutual interest in biosensors and brown water/ocean systems, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on the small scale turbulence of the model ecosystem of the Mississippi River/Gulf of Mexico and the development of biosensors and autonomous platforms for ONR and DOD applications. As a suite of integrated modules, specific projects thus have complemented each other as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

Seven research projects have been conducted in the two primary research modules and have resulted in significant progress related to the overall grant objectives. Assisting in the implementation of the overall project to promote the dual resolution of DOD problems and education of students and the general public was the continuation of four support cores: 1) environmental informatics; 2) computer operations; 3) research support; and 4) communication and education. In addition to the new knowledge developed by the research effort, the program has produced 2 useable technologies for further development, 18 publications and abstracts, 18 presentations, and supported the intellectual development of 2 graduate students, 13 undergraduates, and 6 CBR SPRITE students.

This program reflects the CBR's existing research strengths and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. Transcending traditional structures, the CBR has become a model of academic/government/industry interaction.

List of Acronyms and Abbreviations

AUV	autonomous underwater vehicles
BHRP	Bioenvironmental Hazards Research Program
BMI	Body Mass Index
CBR	Center for Bioenvironmental Research
COTS	Commerical off-the-shelf
DES	diethylstilbestrol
EDCs	endocrine disrupting chemicals
EDTA	ethlenediaminetetraacetic acid
EIC	Environmental Informatics Core
ER	estrogen receptor
GC/MS	gas chromatography/mass spectrometry
GIS	Geographical Information System
GPS	Geographic Positioning System
NAVOCEANO	Naval Oceanographic Office
NGVD	National Geodetic Vertical Datum
NRL	Naval Research Laboratory
SPRITE	Summer Pipeline Research Initiative: the Tulane Experience
TUHSC	Tulane University Health Sciences Center
XU	Xavier University

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Center for Bioenvironmental Research
At Tulane and Xavier Universities

Bioenvironmental Hazards Research Program
Office of Naval Research/US Department of Defense

Annual Productivity Report
August 1, 2002 – July 31, 2003
N00014-99-1-0763

I. OBJECTIVES AND SIGNIFICANCE

The Office of Naval Research (ONR) is interested in long-range science and technology research projects that offer potential for advancement and improvement of Navy and Marine Corps operations and encourages participation by Historically Black Colleges or Universities (HBCUs). This final technical report describes an integrated program of basic and applied bioenvironmental research for technology development, communication and education that supports the ONR Bioenvironmental Hazards Research Program (BHRP). The work described in this report builds upon the DOD's twelve-year, integrated research program (BHRP) conducted at the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities. The CBR focuses on the holistic concept of environmental signaling from molecular to ecosystem levels, with a particular emphasis on development of biosensors, biomarkers, and evaluation techniques related to environmental exposures of human and ecological systems that addresses bioenvironmental problems relevant to the Navy and the Department of Defense (DOD). CBR research taps the basic and applied strengths of two universities that have been directed and refined over a twelve-year period to reflect and, indeed, anticipate DOD environmental research interests.

A. Environmental Priorities of the Office of Naval Research

Many of the DOD environmental programs seek to understand the fate and biological effects of pollutants and contaminants resulting from military operations and training. To achieve this goal, DOD has focused on basic research to understand the biological actions of these agents, biomarkers of exposure, mechanisms of toxicity, and the use of experimental and computational modeling to assess potential health risks. The ONR focuses on "brown water" problems of particular Navy relevance (e.g., major world rivers, estuaries, and continental shelves). Of particular interest to the ONR Biomolecular Science and Technology Program are 1) biomolecular projects related to biosensor development; and 2) biosystems projects related to marine mammal biology and environmental microbiology/engineering.

Central to meeting these challenges is the continued development of new knowledge, technology, and human resources through the nation's universities, where approximately half of the defense science and technology research is currently performed. The ONR is committed to strengthening the scientific capability of colleges and universities with significant enrollments from minorities underrepresented in science and engineering, and providing science-related infrastructure as well as funding for defense research and

engineering programs. Support of the nation's university-based science and education enterprise is an essential component in addressing environmental concerns facing DOD. The CBR submitted a proposal in response to the Broad Agency Announcement (BAA 02-001). In January 2001, the Office of Naval Research awarded the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities a one-year renewal grant of $953,500 in funds for the Bioenvironmental Hazards Research Program (BHRP), beginning in August 1, 2002, through July 31, 2003. This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. This annual productivity report also includes mechanisms for the effective communication of this information for resolution of DOD problems and for the educational training of future scientists.

B. Prior Progress of the CBR Biohazards Research Program

The CBR BHRP to date has developed an integrated approach to increasing the knowledge base of actual and potential impacts on human health and ecological systems of defense-related operations, as well as the processes to restore contaminated environments. To facilitate the long-term security of the Navy, other military services, and the nation, the CBR BHRP has:

- Produced a vast suite of technologies and methods for biohazard monitoring and characterization of ecosystem, wildlife, and human health for application biohazard risk assessments for all of the DOD's branches;
- Developed one of the world's most robust programs for biosensor and biomarker technology development for real-time cost-effective monitoring of heavy metal and organic contaminants and combat-related biohazards in the air, water, and the soil including one deployed on an autonomous underwater vehicle (AUV);
- Facilitated a strategic partnership between academia, military, agency, and the commercial sector in the Gulf South region for a holistic long-term monitoring program for the Mississippi River, the Gulf of Mexico, and airshed to serve as a national testing laboratory for military biohazard monitoring, characterization, and communication;
- Increased the nationwide representation of African Americans with advanced degrees in bioenvironmental fields important to DOD; and
- Funded more than 100 projects over the past decade, including those through the ONR, resulting in hundreds of publications, collaborations, and investments with the commercial sector.

These projects are being conducted through a wide variety of methods including *in vitro*, *in vivo*, epidemiological, modeling, field, and other laboratory studies at Tulane and Xavier Universities and associated sub-contractual institutions. The significance of this research includes a greater understanding of human and ecosystem responses to environmental contamination and their ability to repair or reverse these effects; increased safety for defense

workers and the general public from exposure to toxic exposures; and technologies for advanced monitoring of the environment for the improvement of human and ecosystem health.

An additional benefit of the CBR BHRP is the education and research training provided to minorities. Past research activities have trained both minority faculty and students at Xavier University in scientific analytical techniques and have increased minority representation in these fields. These are techniques applicable to a variety of environmental concerns, but of particular relevance to ONR problems.

The most important result of the CBR BHRP is the development and practical application of basic knowledge and new technologies. BHRP technologies have been demonstrated through numerous environmental applications in both defense and private sectors. Through its research, the CBR has attracted corporate investments including: $1 million from Shell, $75,000 from TRW, and other investments from Uniroyal and Exxon. Many of these BHRP projects have demonstrated either existing or potential commercial and civilian interest or partnerships. The useable technologies that have demonstrated the greatest commercial and civilian interest to date are summarized in **Appendix C**.

C. Current Research Efforts of CBR Biohazards Research Program

This program reflects the existing research strengths of the CBR and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. There are few, if any, academic organizations with this capability. The CBR BHRP research facilitates biomarker, human and ecological health assessment, and biosensor applications to systems that provide useful models for the DOD and ONR.

One module, Environmental Signals and Sensors, utilizes basic research to study how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health; and how biosensors can be developed and utilized for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or to ecological and ecosystem function levels. Given the CBR's and Navy's mutual interest in biosensors and brown water/ocean systems, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on model ecosystem study of the Mississippi River/ Gulf of Mexico and also the development of biosensors and autonomous underwater platforms for ONR and DOD applications. Cores that provide research support include computer operations, environmental informatics, and communication and education to promote the dual resolution of both DOD problems and the education of students and the general public. As a suite of integrated modules, specific projects thus have complemented each other over time as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

II. CBR Capacity

A. Introduction to the CBR

The mission of the Center for Bioenvironmental Research (CBR) ***is to conduct and coordinate research and teaching to enhance global understanding of environmental issues and provide solutions through innovative communication and technology.*** Founded in 1989, the CBR is an innovative and effective partnership between a Historically Black College or University (HBCU) and a major research university that encourages scientists from multiple disciplines to work together to investigate and resolve environmental problems.

Under the leadership of Dr. John McLachlan, Weatherhead Distinguished Professor in Environmental Studies at Tulane University and an internationally recognized environmental scientist and administrator, the CBR has earned a reputation for its scientific research into the environmental problems of Louisiana. In extending its spheres of influence to national and global problems of the environment, the CBR has brought unique focus that reflects a community-based perspective in conjunction with scientific rigor.

CBR programs are organized around five themes:

Partnerships: The CBR integrates faculty and students from Tulane's Schools of Engineering, Liberal Arts and Sciences, Medicine and Public Health and Tropical Medicine and Xavier's Colleges of Arts and Sciences and Pharmacy in innovative ways to optimize interdisciplinary teaching, learning and research.

Human and Ecosystem Health: Integrating diverse disciplines, the CBR has developed a holistic research program focusing on the effects of environmental hormones on humans and ecosystems through the processes of environmental signaling by natural and synthetic hormones and contaminants that mimic those substances.

Water: The CBR has co-evolved its programs on Environmental Signaling and Aquatic Ecosystem Research to create effective connections. Research efforts employ laboratory and field-scale approaches to look at physiochemical, biological, and ecological impacts in the Mississippi River, Gulf of Mexico, and other aquatic and atmospheric ecosystems.

Communication & Technology: The CBR provides research-based knowledge on the origins, interactions and fate of natural and synthetic chemicals in living systems using informatics capacity particularly its data management and GIS teaching lab. Through its web-based information programs and networked digital technologies, the CBR makes complex issues understandable and provides a forum for scientific discourse.

Environmental Education: The pipeline programs provide interdisciplinary training and research opportunities for undergraduate, graduate and doctoral students and faculty. Internet-based educational programs and outreach initiatives strengthen science education on campus and in the local community and region.

B. The CBR Tulane/Xavier Partnership – a Unique Model

Tulane and Xavier Universities have developed a close working relationship in the past thirteen years, aided by a common vision of academic excellence and the development of high quality educational opportunities for minorities and women. The Tulane/Xavier partnership is a well-established, effective joint venture between majority and minority universities. It is an extremely complementary relationship with respect to environmental restoration and waste management, with the Xavier University focus on education and graduate work, and the Tulane University emphasis and experience in education, research, and technology development and transfer. Optimizing the research capabilities of Tulane and the educational resources at Xavier, the reputation of both institutions enhances the ability of the CBR to solicit resources, recruit staff and researchers, sponsor conferences, and execute successful marketing of its education and research programs.

The relationship between Tulane and Xavier Universities is the foundation of the CBR and serves as a working model for all its collaborations. Since the CBR integrates academic structures, it has the freedom to advance teaching and research by creating flexible working groups to address specific needs and problems. Administrators and researchers team up with government, private, academic, and community individuals and agencies to make use of the best intellectual and technological resources. The partnerships catalyzed by the CBR exist at local, regional, and international levels resulting in community-based solutions to environmental health problems.

1. Tulane Capabilities

Tulane University has established itself as a powerful engine of economic development for New Orleans and Louisiana. Beyond that, in its 160-year commitment to education, Tulane has developed itself as a good citizen that provides the spark of creativity and knowledge that attracts and nurtures intellectual talent while directing its resources to the needs of the community. Tulane is the largest private employer in Orleans Parish and ranks 12th in the State of Louisiana.

Since its inception Tulane has grown into one of the nation's premier institutions of higher learning, known widely for both its undergraduate teaching and cutting-edge research. Those achievements are reflected in rankings by national periodicals such as *US News and World Report* that in 2002 ranked Tulane 46th overall among all national universities. Tulane University is ranked among the top 10 private universities in technology transfer and among the top 25 in amount of federal research funding.

The University enrolls a diverse student body of 11,972 students from all 50 states and 100 foreign countries in its undergraduate, graduate and professional schools. The University ranks in the top 10 in Environmental Law Studies, the top 15 in Public Health and International Law, and the top 25 in Biomedical Engineering. Tulane is recognized as both a member of the Association of American Universities and a Carnegie-1 research university, one of only 4 in the South.

2. Xavier Capabilities

Xavier University of Louisiana is the nation's only institution of higher education that is historically African American and Catholic. The ultimate purpose of the University is the creation of a more just and humane society. To this end, Xavier prepares its students to assume roles of leadership and service in society. This preparation takes place in a pluralistic teaching and learning environment that incorporates all relevant educational means, including research and community service.

Xavier University has grown in enrollment in the past 10 years with a current enrollment of 3,912 students. Half of the Xavier students are from Louisiana, while the rest come from 37 other states and 27 foreign countries. The student body is predominantly African American (89%), but the university is open to all. More than half of its students currently major in the natural or health sciences.

The preeminence of Xavier in training undergraduates in science is reflected in its first-place ranking in the placement of African-American graduates in medical schools for the past 9 years. In addition, Xavier is first nationally in the number of African American students earning undergraduate degrees in both biological/life sciences and the physical sciences. From 40 to 47% of all recent graduates enroll in professional and graduate schools. The National Science Foundation has designated Xavier University as one of six to participate in its "Model Institutions for Excellence in Science, Engineering, and Mathematics" program. The Southern Association of Colleges and Schools accredits Xavier University.

C. CBR Support Cores

The state of the art CBR facility at Tulane Health Sciences Center contains sophisticated research equipment including exposure chambers for respiratory disease studies and a Geographical Information Systems (GIS) lab. The facility is electronically networked and physically connected to Tulane Medical School and University Hospital, thus providing easy access between laboratories and clinics. The CBR facility contains state-of-the-art research equipment including a microarray core facility, a fluorescent activated cell sorter, and a high performance gas-liquid chromatograph/dual mass spectrometer. In terms of molecular biologic capabilities, the labs also feature analytical devices, and cellular and molecular biology equipment, tissue-culture facilities, and various containment and decontamination hoods and devices, so that radiation research, protein analysis, PCR, RNA and DNA analyses and immunohistochemical and *in situ* hybridization procedures are routinely conducted in the course of experimentation.

The CBR also has research space on the Tulane University uptown campus. The CBR Uptown contains an additional GIS lab and many equipment cores and lab space for studying neuroscience, molecular biology, and analytical chemistry. The CBR faculty located at Xavier University utilizes modern research and office space in the new seven-module environmental toxicology research center.

1. Research Support

The CBR has a first-rate management team in place with administrative capabilities to develop, implement, facilitate, track, and monitor grants and contracts as well as provide programmatic direction and administrative leadership for the facility. This team includes a director, a deputy director, associate directors, program managers and coordinators, accountants and administrative secretaries. Tulane and Xavier are innovators among universities in facilitating the mechanisms that allow scientists from multiple disciplines to work together in resolving environmental problems. In addition, the organizational structure of the CBR has allowed it to qualify for large integrated, interdisciplinary grants that are beyond the scope of many other universities or research organizations, including individual Tulane and Xavier departments. The work of the CBR is strengthened by this partnership that can provide the faculty, students, and resources that are necessary to conduct the bioenvironmental hazards research projects discussed in this report.

2. Computer Operations

The CBR has established a Computer Operations Core to modernize and facilitate efficient data communication, sample tracking, QA/QC, and data dissemination. Presentation capabilities are enhanced by a state-of-the-art computer operations facility that provides graphics and electronic media-based services and lab-based microscopy with a digital camera and PC with Image Pro archiving and image analysis software.

The Center provides fast, accurate access to the results generated by high tech analytical instruments located in various Tulane and Xavier scientific laboratories. The SGI equipment is primarily utilized for molecular modeling and other supercomputing tasks that require symmetric multiprocessing and geometry engines for advanced 3-D rendering. Apple and PC notebooks enable the CBR staff to communicate with the department by establishing a link for Internet access, electronic mail, and data transfers while attending events away from the home base. In addition to the equipment currently maintained at satellite locations, the CBR has shared access to Tulane University distributed computing environment of high performance RISC computers. These machines enable the Tulane community to stay connected with other institutions that are part of Internet 2 at data transfer speeds in excess of 100 Mbit/sec as well as other Internet entities at 10Mbit/sec.

During the ONR project period, the Computer Operations Core established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange. Collaborative efforts were completed in the establishment of multi-platform wireless connectivity on and off-campus. As part of the effort to maintain these services the Computer Operations Core acquired various hardware and software, implemented wireless networking protocols, managed web-based information exchange, and instituted large monitors for demonstration of research concepts and web editing tools. The Core researched the effectiveness of remote conferencing among researchers for virtual meetings.

3. Environmental Informatics

During the ONR project period, the Environmental Informatics Core provided spatial (GIS) analysis, remote sensing, mapping and other cartographic product development in collaborative projects with BHRP researchers. The Core validated the underlying causes behind the patterns of bathymetric change discovered and measured in previous years of ONR funding. This work resulted in collaboration with researchers at the University of Louisiana at Lafayette and the University of New Orleans in developing a hydro-ecological model for the lower Mississippi River. Additionally, the EIC continued its research support on the habitat characteristics of the *Aedes aegypti* and *Culiseta melanura* mosquitoes. Major accomplishments included:

- Validation of historic trends in the bathymetry of the lower Mississippi River by including an 1893 dataset in the study, which verified that the riverbed below New Orleans is eroding, and the riverbed between New Orleans and Baton Rouge is aggrading.
- Determination of correlations between key mosquito species and habitat characteristics, finding that: (1) the denser the canopy, the taller the trees, the more pines, the more hardwoods, and to a lesser extent the more undergrowth, the higher the mosquito populations; (2) mosquitoes avoided non-forested areas, particularly grassy areas in the summer months; and (3) there were higher mosquito populations in lower elevations, closer to bayous, and in water-collecting areas. These and other findings were presented to the Entomological Society of America and elsewhere.

4. Communication and Education

Funds were allocated from the Research Support core to cover student support for research teams. In this way the CBR could assist research faculty to build and train sufficient qualified personnel to complete research and also create capacity in undergraduate students. Funds were also provided for three projects in the Communication and Education core for training of students through educational initiatives. They were: 1) two education pipeline pilot programs; and 2) the annual international symposium of presentations, posters and workshops on environmental signaling (the e.hormone conference).

Education Pipeline Pilot Program: With ONR support, the CBR continued the successful SPRITE program as part of its undergraduate education pipeline initiative to increase the number of African American students enrolling in graduate science programs. The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) provided Xavier undergraduate students a graduate-level laboratory research experience and mentored introduction to graduate life. In summer 2002, six students were competitively selected from a pool of 25 applicants for the 10-week summer program. An additional three Xavier University students were involved as Ronald McNair Scholars in the program.

The summer 2002 program involved faculty research mentors from Tulane University Departments of Civil and Environmental Engineering, Medicine/Hematology and Oncology, Microbiology/Immunology, Ophthalmology, Pathology, and Tropical Medicine. In spring 2003, two of the six competitively selected students were accepted to post baccalaureate schools/programs. Of the two, one was accepted to Louisiana State

University Medical School, and the other was accepted into Xavier University College of Pharmacy. The remaining four students were either still applying to various post-baccalaureate programs or still had one more year of undergraduate schooling before submitting such applications. In four years, this program has become a major pipeline of minority students to Tulane graduate and health professional schools.

International Environmental Hormone Symposia: One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is the impact of bioenvironmental contaminants on the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (eg. contaminants and pollutants) and its effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. For the past four years, the annual Environmental Hormone Symposium (*e.hormone*) in October initiated and hosted by the CBR has been a national and international focal point for all those who are interested in the field of environmental signaling.

The goal of the *e.hormone* symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on environmental signalling and contribute to the future of this field. The symposium format includes scientific presentations grouped around conceptual themes. Preeminent experts in the field introduce sessions and provide historical perspective on their topic and highlight recent findings. Presentation topics range from human to ecosystem health and from basic research to population studies and all explorations were at the cutting edge of research and policy. The primary strength of this annual forum of information exchange and collegial interaction is its multidisciplinary and multinational nature. Each of the past four symposia has been reported on the web, and its scholarship recognized in publications such as *Science News* and *the Annals of the New York Academy of Sciences*. *e.hormone 2002* was host to 36 speakers (9 international) and 128 participants (39 international).

In summary, the CBR is dedicated to training students for careers in science. The CBR sponsors numerous programs to increase and enhance undergraduate, graduate, and faculty research and training opportunities at Tulane and Xavier Universities. A primary CBR goal is to encourage and enhance minority participation and representation in the sciences. The Communication and Education core reflects a collection of enriching environmental education programs that promote awareness of pertinent issues, offer interactive encounters between young and veteran scientists, and provide career-building research experiences in bioenvironmental, biomedical, and environmental sciences.

III. OVERVIEW OF RESEARCH

A. Introduction

Research associated with environmental problems of importance to the DOD has required an integrated approach from fundamental science to communication of research results. This report provides a set of integrated research modules that will continue the CBR/ONR BHRP

partnership and serve as a model for other DOD research along environmental lines. The CBR has earned a reputation for its scientific research into relevant environmental problems, while becoming a model of academic/government/industry interaction. Transcending traditional academic structures, the CBR provides a powerful tool for modernization of teaching and research.

These research projects integrate common research themes of environmental signals and ecosystem monitoring as well as common technologies of biosensors or biomarkers. Computer Operations and Environmental Informatics cores ensure integration of this research in conjunction with Communication and Education core, which elucidates communication of this research and the flow of information to future scientists and engineers. One of the benefits of this modular approach is the applicability of these approaches to environmental problems using research themes or technologies that are common across platforms. An additional benefit of these reports is the provision of a mechanism for the bi-directional flow of initiatives and insights from the Navy to and from academia, while providing for the education of future scientists.

In the area of Environmental Signals and Sensors, five projects were funded. Of these five, one was a joint Xavier/Tulane effort, led by the Xavier investigator. In the area of Ecosystem Monitoring and Assessment, two projects were funded. Of these seven projects, Xavier investigators led four. In the Communication and Education area, two projects were funded. One was a joint Tulane/Xavier effort. A total of nine (9) research projects were funded under the CBR BHRP award of June 2002.

B. Environmental Signals and Sensors

A principal goal of our ongoing research is to examine the actions of DOD-relevant contaminants/pollutants (e.g., organo-chlorine compounds, PAH's, and heavy metals) on important cell targets. Long-term goals of the research in this module are to identify suitable biological markers that will serve as early indicators of toxicant exposure in humans and wildlife and potentially of the overall health of the ecosystem, thereby linking with the other research module in this project. The research addressing this theme will elucidate a scientific basis to develop rational biologically based risk assessment models.

The projects related to this theme apply molecular biology to elucidate mechanisms of toxicity of these signals and to develop new methods for sensing of toxicants in the environment. Understanding the mechanisms of these toxic reactions permits the development of real understanding of the hazard posed by various contaminants. The data derived from these projects will yield scientific bases to assess risk to humans and wildlife.

In the area of **Environmental Signals and Sensors,** five (5) projects were funded.

- Dr. Matthew Burow, Assistant Professor of Medicine, with Co-Investigator Dr. John McLachlan, Professor of Pharmacology, Tulane University, is developing technologies and methods to identify a basic mechanism by which selected environmental agents subvert the estrogen and cell survival signaling pathways, thereby leading to potential developmental defects and/or disease states ("*Effects of*

Estrogens and Endocrine Disrupters on Suppression of Apoptosis in Normal and Neoplastic Breast Epithelial Cells").

- Dr. Valerie Wilson, Clinical Associate Professor of Environmental Health Sciences, Tulane University, is evaluating the role of biologic and environmental factors related to regulation of uterine function in women ("*Human Health Applications*").
- Dr. Shubha Kale Ireland, Associate Professor of Biology, Xavier University, is testing the effects of low-level exposures of certain commonly found environmental pollutants, particularly heavy metals, on the frequencies of L1 retrotransposition ("*L1 Retrotransposition: A Biomarker for Exposure to Low-levels of Environmental Pollutants*").
- Dr. Tanya McKinney, Assistant Professor of Biology, Xavier University, is studying phenotypically and genotypically gram negative (fecal and non-fecal) bacteria in the lower Mississippi River that are resistant to a panel of commonly used antibiotics (*"Identification and Characterization of Antibiotic Resistant Riverine Gram Negative Bacteria"*).
- Dr. Robert Blake, Professor and Chair of Basic Pharmaceutical Sciences, Xavier University, and Co-Investigator Dr. Diane Blake, Professor of Biochemistry, Tulane University, are developing biosensors that will permit the rapid automated identification and quantification of environmental contaminants ("*Characterization of Novel Antibodies for Autonomous Underwater Vehicles*").

C. Ecosystem Monitoring and Assessment

The Navy requires a fundamental understanding of fate, transport and transformation effects of contaminants in estuarine and near-shore environments. Since DOD operations can frequently result in the release of a variety of perturbations in a region, a holistic assessment of potential biohazard impacts must include ecosystem-level analyses. Through basic and applied research, the CBR is developing sensor devices that will monitor potential and actual exposure of troops in the field to harmful chemical or biological agents. CBR research emphasis is on providing innovative cost-effective solutions to prevent, minimize, or remedy human health or ecological hazards. CBR expertise on environmental characterization, monitoring, and assessment achieved provides an essential segue into practical research on appropriate environmental impact assessments and management based on that assessment. Research results will advance the current state of knowledge in remediation policies (including determinations of self-remediation) and can result in substantial cost savings for the DOD and other public and private entities in future environmental impact assessment efforts.

In the area of **Ecosystem Monitoring and Assessment** two (2) projects were funded.

- Dr. Michael Adams, Associate Professor of Chemistry, Xavier University, is studying whether appropriate modification of bridging phosphido ligands in the diiron complexes will significantly increase the solubility of these compounds in environmentally friendly solvents ("*Synthesis of Diiron Complexes Containing New Phosphido-bridging Ligands*").

- Dr. Douglas Meffert with Co-Investigator George Rey, President of COTS Technology, LLC, are developing predictive tools that will provide local, regional, and national policy/decision makers with decisive information that can be applied to monitoring the risk of exposure to defense related toxicants by using the Mississippi River/Gulf of Mexico estuary system as a model ("*River Communication*").

IV. SUMMARY ACCOMPLISHMENTS

A. Overview

Accomplishments on this grant can be documented in three major areas:

1. ***Research Publications, Abstracts and Presentations*** – that document progress made in the research through publication in a number of peer-reviewed venues available to the general scientific community;
2. ***Development of Useable Technologies*** – which have direct benefit on furthering the mission-related scientific interests of ONR; and
3. ***Intellectual Development***- that extends the ability of the grantee and ONR by developing the next generation of scientists.

For final investigative research reports, see Appendix A.

Research Publications, Abstracts and Presentations:
Research from this project period resulted in the following publications in scientific journals and conference reports:

- Sixteen (16) publications in research journals and in conference reports such as *Biochemistry, Carcinogenesis, Environmental Health Perspectives, International Journal of Oncology, and Journal of Steroid Biochemistry and Molecular Biology* to name a few.
- One (1) published abstract in meeting and symposium reports.
- Eighteen (18) presentations at major scientific conferences across the country and internationally including, but not limited to, the annual meetings of the American Chemical Society, American Association for Cancer Research, Entomological Society of America, e.hormone 2002, Marine Technology Society, and the Institute of Electrical and Electronics Engineering.

A complete listing of the publications, abstracts and presentation made by investigators on this project can be found in Appendix B.

Development of Useable Technologies:
Research results from this project are generating two useable technologies.

One technology that is part of the Environmental Signals and Sensors area is:

- Immunosensor for AUV Deployment. This antibody-based biosensor will automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy. A self-contained, automated

immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters. An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument. Sapidyne Instruments (Boise, ID) is constructing the immunosensor and the Tulane laboratory, in conjunction with the Naval Research Laboratory (Dr. Fran Ligler, Washington, DC), is working closely with them to coordinate the development of biological reagents with the development of the instrument. A provisional patent application entitled "Recombinant antibodies that bind to metal-chelate complexes" was filed in March 2001.

The one technology that is part of the Ecosystem Monitoring and Assessment area is:

- Integrated Autonomous Immunosensor & Autonomous Underwater Vehicle (AUV) System. This system will enhance real-time biosensor deployment for environmental compliance and ultimately biologic warfare detection. The projected timetable for the completion of AUV/ biosensor integration is August 2004, and the biosensor will be deployed on AUV or stationary buoys and AUVs subsequent to August 2004, pending acquisition of REMUS AUV. Partners are Tulane and Xavier Universities; COTS Technology, LLC; Sapidyne Instruments (ID); Woods Hole Oceanographic Institute (MA); and the US Naval Oceanographic Office (MS). The partners will apply for patents.

Further details on the potential technology products can be found in Appendix C.

Intellectual Development:

The research effort provided for the intellectual development of the faculty who participated in the project. In the process of conducting their research, investigators collaborated with new and existing partners in research, and at times formed unique consortia and research teams. Several of the investigators worked across departmental boundaries and in a few instances, faculty members from each component university formed a Tulane/Xavier research team to undertake a project.

The project has supported the research work of:

- 2 graduate students working with Tulane investigators.
- 13 undergraduate students, including 12 Xavier undergraduates working with Xavier investigators, and 1 Tulane undergraduate who worked on projects with a Tulane advisor. These undergraduates conducted research with a variety of investigators and thus were exposed to a variety of aspects of the overall project.
- 6 SPRITE (undergraduate) students who were chosen out of an applicant pool to follow a mentored graduate-level laboratory research experience and gave a scientific presentation of their research project results to mentors, faculty, and peers at the conclusion of the summer program.

A complete listing of the student names and principal investigators/advisors can be reviewed in Appendix D.

B. Environmental Signals and Sensors

- Ability of estrogenic chemicals (estradiol, DES, DDT) to exert effects on cell survival pathways of breast carcinoma cells required an intact ERK-MAPK-pathway; DDT can also function to induce apoptotic pathways at higher doses.
- Specific organ chlorines can activate a number of other signaling pathways including antioxidant response elements, estrogen response elements (ERE), hypoxia-induced factor (HIF) and cyclic AMP response element (CRE).
- Ability of organ chlorine to activate MAPK and potentially mediated cell survival/death signaling in human tissues is supported by further study demonstrating that activation of p38 signaling can promote cell survival and therapeutic resistance in breast carcinoma cells.

- Surgical chart data of women receiving uterine fibroid treatment at Charity Hospital or from a private physician affiliated with this project indicated that a major shift in age of menarche occurred in women 44 years of age or younger, as compared to women 45 years or older
- None of the risk factors examined in these studies appeared to be significant predictors of multiple uterine fibroids, though BMI (a measure of obesity) was nearly significant.
- The decline in the age of menarche in the late1950 or early1960's may implicate an environmental factor (yet to be defined) in the condition of uterine fibroids.

- A transient assay for L1 frequency measurement, which is effective but can be completed in a short period of time (4 weeks), was developed; the transient assay uses human (HeLa) cells as opposed to the original assay, which uses mouse fibroblast (3T3) cells.
- Using filtered HgS, a known carcinogen with low solubility in water, L1 transient assay showed significant increases in the L1 retrotransposition, substantiating other data linking increased L1 jumping to human diseases including certain types of cancers.

- Of the 611 gram negative isolates isolated from 4 sites during this project period, 103 have been identified; the major genera of these bacteria were *Aeromonas, Chromobacterium,* and *Pseudomonas.*
- The majority of the isolated organisms were resistance to ampicillin and at least one other antibiotic (streptomycin, erythromycin, tetracycline, ciprofloxacin, or Bactrim).
- Of the 23 *Aeromonas hydrophilia* strains studied, only one had an observable plasmid.

- Antibody 5B2 exhibits a wide range of allosteric behaviors.

- Other antibodies, such as 2D42, bind their antigen with positive cooperativity that is characterized by a Hill coefficient of greater than two.
- As the first detailed descriptions of allosteric binding behavior by antibodies, these observations provide new insights into a fundamental property of antibody functional behavior that appears to have been largely unnoticed.

C. Ecosystem Monitoring and Assessment

- New diorganochlorophosphines, PR_2Cl, bearing long perfluorinated organo substituents produced via reaction of the Grignard $CF_3(CF_2)_6CH_2CH_2MgI$ with PCl_3.
- Changes in reaction conditions aimed at optimizing the yield of the desired phosphine, $[CF_3(CF_2)_6CH_2CH_2]_2PCl$, and avoiding production of $PRCl_2$ were explored.

- Laboratories dedicated to long-term river, gulf, ocean and water research, and related biomolecular sciences, leveraging upon CBR BHRP successes for the ONR, are being developed to accomplish projects chosen by scientists working with industry, using state-of-the-art research and experimentation technology.
- Significant regional issues with national relevance, including pollution, climate, river degradation, coastal erosion and subsidence, and water supply, are being explored for RiverSphere implementation.
- Program for long-term communication of its programs, translating basic science into applications supporting naval operations and educating and informing the public, was initiated and made available to audiences.

APPENDIX A.

INVESTIGATOR RESEARCH REPORTS

Computer Operations Core

Principal Investigator: John Vassilopoulos, MS
Director
Computer Operations
Center for Bioenvironmental Research

Reporting Period: August 2002 – July 2003

Primary Objectives of Research Activities

To provide end-user technical direction for data warehousing, networking, securing storage for project-related data, and to coordinate with research support efforts for all CBR core projects.

Progress Made to Achieve these Objectives

- Hardware and software acquisitions for security and backup procedures.
- Implementation of wireless networking schemes.
- Software for CD/DVD publishing.
- Large monitors for demonstration of research concepts and web editing tools.
- Web-based information exchange.

Major Accomplishments (bullet format)

- Established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange.
- Completed collaborative efforts in establishing multi-platform wireless connectivity on and off-campus.
- Researched effectiveness of remote conferencing among researchers for virtual meetings.

Publications, Manuscripts, Abstracts

N/A

Presentations

N/A

Intellectual Development

None

Useable Technologies

N/A

Environmental Informatics Core

Principal Investigator: Douglas J. Meffert, Ph.D.
Deputy Director
Center for Bioenvironmental Research
At Tulane and Xavier Universities

Co-Investigator: Richard Campanella, M.S.
Assistant Director
Environmental Analysis & GIS/Remote Sensing Specialist
Center for Bioenvironmental Research

Reporting Period: August 2002 – July 2003

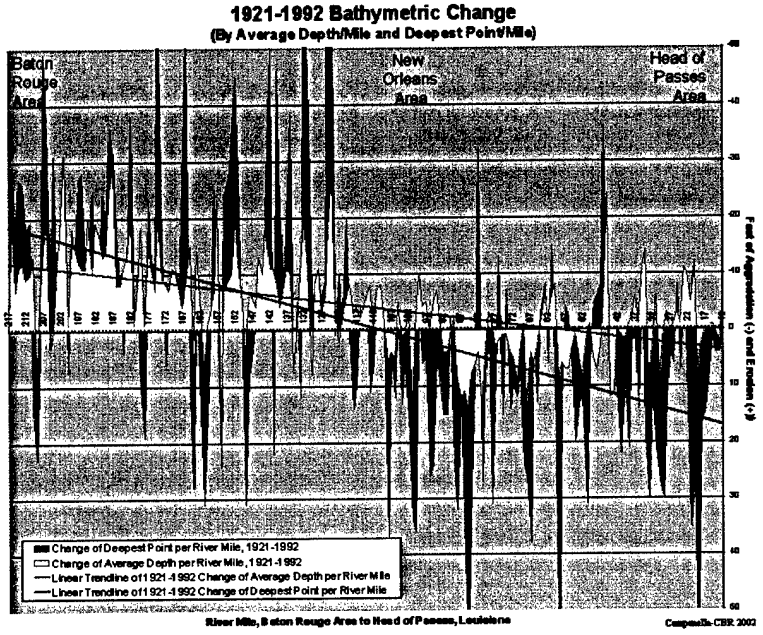
Primary Objectives of Research Project

The Environmental Informatics Core (EIC) proposed for this reporting period to validate and investigate further the underlying causes behind the patterns of bathymetric change we had discovered and measured in previous years of ONR funding. We succeeded in validating the patterns, and have used our experience in this project to collaborate with researchers at the University of Louisiana at Lafayette and the University of New Orleans to develop a hydro-ecological model for the lower Mississippi River. Additionally, the EIC proposed to conduct research on the habitat characteristics of the *Aedes aegypti* and *Culiseta melanura* mosquitoes (in collaboration with Dr. Dawn Wesson) in southeastern Louisiana. Finally, the EIC proposed to support other cores and ONR-related CBR activities with GIS, remote sensing, and the development of cartographic products as needed.

Progress Made to Achieve these Objectives

Analysis of bathymetric change aids in the understanding of controlled rivers and their relationship to the estuary. But historical bathymetric data are either not readily available or are not held to the same geodetic standards as more recent data. Surveys conducted in 1921, 1937, 1948, 1964, 1975, 1983, and 1992 were recently been digitized and released by the New Orleans District-Army Corps of Engineers. Prior to and during the 71 years spanned by these surveys, the river has been extensively modified for flood control, while the river valley has been developed. These bathymetric surveys can be used to examine the river's response to these modifications of the drainage basin, river channel, and levee system. For this analysis, these historical bathymetric datasets were assembled from various formats, geo-referenced, adjusted to account for differences in vertical datums and stage, corrected for errors, interpolated, and differenced for both average depth and deepest point, at the one-river-mile level. In the summer of 2002, the Environmental Informatics Core processed the 1893 bathymetric survey points for the study area by georeferencing historic maps and heads-up digitizing the deepest point per mile and its depth attribute. These data (as well as the 1921 data) were then adjusted to the same vertical datum (NGVD 1929) as the later datasets, a calculation that required a significant amount of research to determine. This adjustment was critical because the 1921 and 1893 datasets were based on antiquated vertical datums (such as the Memphis Datum of 1880), and could not be compared to

later datasets based on the National Geodetic Vertical Datum of 1929. The results further supported the trend we had detected in the period 1921-1992: that the lower 100 miles of the river (below New Orleans) have generally eroded, while miles 100-200 (new Orleans to Baton Rouge) have generally aggraded, and that these trends were especially pronounced in the first half of the time period. Results were presented at a number of professional forums, as listed below. Additionally, the results proved relevant to a study conducted by J.J. Galler, T.S. Bianchi, M.A. Allison, and L.A. Wysocki of the Tulane Department of Earth and Environmental Science, entitled "Biochemical Implications of Levee Confinement in the Lowermost Mississippi River." This is scheduled for publication in EOS Transactions in the upcoming months. Richard Campanella will be a co-author.



Graph above: Analysis of *average* bathymetric change at the river-mile level from 1921-1992 shows that the lower Mississippi River has been aggrading from the New Orleans area up to the Baton Rouge area (miles 100-200), and eroding from New Orleans down to Head of Passes (miles 100-0), at a rate of roughly one foot per 10 river miles, with most change occurring in the 1921-1948 period. When the change of the *deepest* point is analyzed per river mile (1893-1992), the aforementioned trends are more pronounced. The data also show that channel changes in the X-Y direction from New Orleans to Baton Rouge varied to a slightly greater degree than from New Orleans to Head of Passes, which concurs with the bathymetric observations. The previous year of funded research allowed us to add the important 1893 dataset to this study.

Progress in researching habitat characteristics of *Aedes aegypti*, *Culiseta melanura*, and other mosquitoes (in collaboration with Dr. Dawn Wesson) in southeastern Louisiana included the development of a GIS-based mechanism to correlate the distributions of these insects (as determined through traps arranged throughout two study areas near New Orleans) to landscape characteristics. Results indicate that most species consistently preferred forested areas throughout the study period, with some month-to-month variation probably due to other factors. The denser the canopy, the taller the trees, the more pines, the more hardwoods, and to a lesser extent the more undergrowth, the higher the mosquito populations. Urban areas were studied separately, and showed weaker correlations than species in rural areas. These results were presented at two professional forums, as reported below.

Major Accomplishments

- Validated historic trends in the bathymetry of the lower Mississippi River by including an 1893 dataset in the study, which verified that the riverbed below New Orleans is eroding, and the riverbed between New Orleans and Baton Rouge is aggrading.
- Contributed these data to a peer-reviewed article in EOS Transactions, scheduled for publication.
- Used the above study to collaborate with researchers at the University of Louisiana at Lafayette and the University of New Orleans to develop a hydro-ecological model for the lower Mississippi River. This project was recently funded.
- Determined correlations between key mosquito species and habitat characteristics, finding that: (1) The denser the canopy, the taller the trees, the more pines, the more hardwoods, and to a lesser extent the more undergrowth, the higher the mosquito populations; (2) mosquitoes avoided non-forested areas, particularly grassy areas in the summer months; and (3) there were higher mosquito populations in lower elevations, closer to bayous, and in water-collecting areas. These and other findings were presented to the Entomological Society of America and elsewhere.

Publications (Manuscripts and Abstracts)

Galler, J.J., T.S. Bianchi, M.A. Allison, L.A. Wysocki, and **R. Campanella**. "Biochemical Implications of Levee Confinement in the Lowermost Mississippi River," *EOS Transactions, American Geophysical Union.* In press; scheduled for publication in late 2003.

Presentations

Patterns of Bathymetric Change in the Lower Mississippi River, 1893-1992. Presented in various increments at the Governor's Office of Coastal Affairs' "River Resources" Workshop on February 20, 2003 in New Orleans; Army Corps of Engineers "Comparing Rivers: The Mississippi and the Niger" Conference on November 7-8, 2002, in New Orleans; the Environmental Research Consortium of Louisiana (ERCLA) "Environmental State of the State Conference" Conference, October 11, 2002, in Lafayette; the Louisiana Remote Sensing-Geographic Information Systems Conferences, April 2002, in Baton Rouge. Research co-authored by Dr. Bernard Coakley.

Habitat Characteristics of Potential Arbovirus: Field Observations in St. Tammany Parish, Louisiana. Presented in increments at the Louisiana RS/GIS Conference, April 29-30, 2003 in Lafayette, Louisiana; and Entomological Society of America Conference, November 17-20, 2002, in Fort Lauderdale, Florida.

Intellectual Development

N/A

Useable Environmental Technologies

None

Partners (academia, industry, labs/centers, federal agency, etc.)

University of Louisiana at Lafayette and the University of New Orleans

Patents (applied for and issued)

None

Communication and Education Core

SPRITE: The Summer Pipeline Research Initiative: the Tulane Experience
A Mentored Introduction to Programs of Study for Graduate Research

Principal Investigator: Valerie Petit Wilson, Ph.D. *formerly*
Clinical Associate Professor, Environmental Health Sciences
Deputy Director, Center for Bioenvironmental Research
at Tulane and Xavier Universities

Co – Investigators: Charles E. Allen, III, MSPH
Education and Outreach Coordinator
Center for Bioenvironmental Research

Dana M. Greene-McDowelle,Ph.D.
Assistant Professor, Biology
Xavier University of Louisiana

Reporting Period: August 2002 - July 2003

Primary Objectives of Research Activities

The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) is an educational initiative that the Center for Bioenvironmental Research (CBR) coordinates between Tulane University's (TU) Molecular and Cellular Biology (MCB) Graduate Program and Xavier University's (XU) Office of Sponsored Programs. The goal of SPRITE is to increase the number of minorities at the graduate level in the bioenvironmental and biomedical sciences by:

- Providing Xavier undergraduate students with a quality bench research experience in an MCB laboratory under the guidance of an established researcher; and
- Exposing these students to graduate life and the MCB program of Tulane University.

The program focuses on two unique resources: Tulane's successful biomedical graduate program and Xavier University's outstanding pool of science majors. The intent of the program is to provide a mentored introduction to Tulane's excellent programs of graduate study with a successful research experience partially supported by ONR.

In addition to the research, students also participate in weekly seminars and roundtable discussions led by the SPRITE program staff. These seminars cover areas such as research at the frontiers of science, financial aid and career opportunities. Other sessions include forums on the presentation of a research seminar and training on graphics and presentation skills.

The culminating event for the summer activities is a research symposium in which student interns present their work to the assembled faculty, students, laboratory colleagues and staff. Subsequently, in the following fall, students have access to SPRITE staff for assistance in applying to graduate and professional schools for the appropriate academic year.

Progress Made to Achieve These Objectives

For summer 2002, program coordinators developed application packets and disseminated information to eligible sophomores, juniors, and seniors at Xavier University. Presentations were made to faculty and students at Tulane and Xavier Universities to recruit both mentors and research interns.

In summer 2002, six students were competitively selected from a pool of 25 applicants for the 10-week summer program. An additional three students were involved in the program. All three were students of Xavier University and were involved in the school's Ronald McNair Scholars Program. All had their own program stipends and needed only to have structured research experiences.

The summer 2002 program involved faculty research mentors from the following disciplines: Civil and Environmental Engineering, Medicine/Hematology and Oncology, Microbiology/Immunology, Ophthalmology, Pathology and Tropical Medicine

Activities by the SPRITE coordinators in fall 2002 included assistance with student applications to graduate school and other professional schools.

In spring 2003, two of the six competitively selected students were accepted to post-baccalaureate schools/programs. Of the two, one was accepted to Louisiana State University Medical School and the other was accepted into the Xavier University College of Pharmacy. The remaining four students were either still applying to various post-baccalaureate programs or still had one more year of undergraduate schooling before submitting such applications.

Selection of SPRITE interns for the summer 2003 program was equally competitive; 20 applications were received for the 6 positions. Funding for this set of interns was provided in total by 2002 ONR funding.

The success of SPRITE provides a national model for pipeline partnerships between research institutions and historically black colleges/universities.

Publications

None

Presentations

By Students – First, the 2002 interns presented at the CBR's summer undergraduate research academy symposium, which was held on Friday, August 5, 2002 at the CBR.

By Program Coordinators- A poster was presented at the 2003 Annual Biomedical Research Conference for Minority Students held in New Orleans, LA on November 13-16, 2002. The poster, which was presented, highlighted all CBR programs along with SPRITE, which are part of the CBR's suite of programs known as the Environmental Education Pipeline.

Intellectual Development

1. Student Name: Dare' Adewumi
2. Funding Period: May through August 2002
3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled *"The Role of Interferons in the Inhibition of Herpes Simplex Virus Immediate Early Promoters"*
4. Research Advisor: Dr. Aline Scandurro, Microbiology and Immunology, TUHSC

1. Student Name: Angie Curtis
2. Funding Period: May through August 2002

3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled *"The Role of the Distal Bone in Mouse Digit Regeneration"*
4. Research Advisor: Dr. Ken Muneoka, Cell and Molecular Biology, TUHSC

1. Student Name: Renada Fruga
2. Funding Period: May through August 2002
3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled *"The Role of p300/CBP in p53's Transcriptional Regulation of PCNA"*
4. Research Advisor, Dr. Gilbert Morris, Pathology, TUHSC

1. Student Name: L'Issa Gates
2. Funding Period: May through August 2002
3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled *"Induction of T cell Immunity by Dendritic Cells"*
4. Research Advisor: Dr. Mansour Mohamadzadeh, Hematology and Oncology, \ TUHSC

1. Student Name: Erin Humbles
2. Funding Period: May through August 2002
3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled, *"Cloning and Sequencing of a gene encoding topoisomerase IV of Vittaforma Corneae"*
4. Research Advisor: Dr. Paul Brindley, Tropical Medicine, TUHSC

1. Student Name: Maiysha Jones
2. Funding Period: May through August 2002
3. Duties and Responsibilities: Student was responsible for conducting research on the project entitled *"Affect of Alkalinity on Sorption of Naproxen on Activated Carbon"*
4. Research Advisor: Dr. Glen Boyd, Civil and Environmental Engineering, TUHSC

Useable Technologies
N/A

Symposium on the Environment & Hormones (e.hormone), 1999 - 2002

Principal Investigator: John McLachlan, Ph.D.
Weatherhead Distinguished Professor and Director
Center for Bioenvironmental Research
at Tulane and Xavier Universities

Reporting Period: August 2002 – July 2003

Primary Objectives of Research Project:

One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is the impact of bioenvironmental contaminants on the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (e.g. contaminants and pollutants) and effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. This becomes a daunting task since the scientific terminology and methodology, the meetings attended, and literature read by researchers does not usually overlap. The CBR responded to the need for a scientific forum for information exchange and collegial interaction for scientists involved in environmental signaling research by hosting the first international Symposium on the Environment and Hormones (e.hormone) in October 1999. e.hormone has become an annual event.

The goal of this symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on environmental signaling and contribute to the future of this field. The fourth annual e.hormone symposium took place in New Orleans, October 17-19, 2002. It was a multidisciplinary, multinational event. Topics ranged from human to ecosystem health, from basic research to population studies. As always, it was an active meeting with lively discussions of the hottest issues; formal and informal networking opportunities were built into the schedule of symposium activities.

e.hormone 2002 was host to 36 speakers (9 international) and 128 participants (39 international). Ecologists, chemists, endocrinologists, toxicologists, zoologists, engineers, philosophers, undergraduate science faculty, high school teachers, policy makers, and media from the United States, Japan, Europe, and Latin America came together to analyze the latest findings on environmental signaling that are the basis of endocrine disruption. Explorations remained at the cutting edge in research and policy.

After attending this continuing education activity, the participant should have been able to:

- Interpret cutting edge research and techniques related to environmental signaling and apply current knowledge to future research or decision-making
- Identify research interests and colleagues in the field of environmental signaling that will foster future collaboration or information exchange
- Understand philosophical approaches, concepts, frameworks, and policy implications related to endocrine disrupting chemicals and environmental signaling
- Comprehend the etiology of endocrine disorders such as breast and uterine disease, early puberty, and abnormalities in sexual development and functioning.

Progress Made to Achieve these Objectives

e.hormone has become the focal point for all those who are interested in environmental signaling. Sessions were held at the CBR conference facility in the Health and Environmental Research Building in downtown New Orleans. Participants felt the organization of the program, with several talks on a topic was a welcome antidote to the usual dizzying fragmentation of subjects at conferences. The organizing committee, cognizant of the latest literature and findings, selected presenters who conduct cutting edge research in a variety of disciplines and represent diversity in race/ethnicity, gender, geography, and senior/junior research status. This was an additional strength of the carefully constructed program; it did not merely feature the "usual suspects" – eminent investigators that many in the audience had already heard numerous times; fresh voices with interesting new work were welcome. In particular, the 2002 program featured two very well received graduate student presentations, devoted time to exploring the global and historical nature of the field, and hosted two related workshops to increase communication.

e.hormone 2002 featured exciting presentations and information exchanged in a collegial atmosphere. The theme of signaling across organisms and the susceptibility of numerous systems to the deleterious effects of environmental hormones was also an effective organizing principle. In general the topic sessions, representation from European and Asian scientists, and the caliber of the talks resulted in a highly productive and enjoyable meeting for all participants.

Examples of ONR-related research topics and themes at e.hormone 2002 include the following presentations: Tyrone Hayes (University of California, Berkeley) *the Common Pollutant, Atrazine, Alters Sexual Development of Male Frogs*; Caren Helbing (University of Victoria, British Columbia) *Thyroid Hormone Signaling: A Lesson from Frogs*; Susan Jobling (Brunel University, UK) *Why Won't Boy Fish Be Boy Fish?*; Jose Vargas (University of Costa Rica) *Ecological Considerations in Invertebrate Health; Results from a Costa Rican Coastal Pollution Survey*; Dana Kolpin (USGS) *Inventory of Pharmaceutical Chemicals and Other Biologically Active Pollutants in US Streams and Rivers*; Thomas Wiese (Xavier University) *Molecular Determinants of the Estrogen, Androgen, and Progestin Activities of Environmental Hormones*; Robert Twilley (University of Louisiana at Lafayette) *From Environmental Hormones to Ecological Habitats: Defining Models of Ecosystem Self Organization.*

Major Accomplishments

Throughout its four-year history, the e.hormone symposium has resulted in the creation of an extensive global network. Major accomplishments include:

- Diversified participation, and symposium continuity – participants came from 15 countries outside the US; many returning attendees as well as new faces in 2002
- Comprehensive poster session for junior investigators - 40 posters
- A global approach to science and communication via two conference workshops:

  Workshop 1: The Minamata-New Orleans Workshop on Environmental Mercury explored the 50-year case study of Minamata disease in which public health activity, informed by endocrine disrupter research, has influenced public policy in Japan. The goals of the Minamata Workshop were to: explicate the history of Minamata disease, analyze and apply

lessons learned in relation to current policy in Japan, explore mechanisms of mercury-associated developmental disorders, consider a new generation of innovative biosensors for the detection of environmental mercury, and initiate an ongoing dialogue on policy making issues.

Workshop 2: Ecosystem Communication: Global Networks on the Environment attempted to increase participation of Latin American scientists in the "e.hormone network." The goals of this workshop were to: gather scientists from different fields to discuss new approaches to ecological communication, with an emphasis on endocrine disruption across biological systems; and initiate discussion and collaboration on multi-disciplinary topics that will link ongoing research in the US, Europe, and Latin America.

- Maintenance of the "spin-off" e.hormone website as a hub of scientific and media information connecting research colleagues throughout the year

Publications (Manuscripts and Abstracts)

Each of the past four symposia has been reported on the web, and its scholarship recognized in publications such as *Science News*.

Presentations

e.hormone 2002 sessions:

I	Hormones and the Environment: A Global Perspective
II	Cell Signaling and Hormone Action: The Emerging Paradigm
III	Ecological Signals
IV	Chemical Approaches to Endocrine Disrupting Chemicals
V	The Future of the Field
VI	In Vitro & In Vivo Model Systems for Studying Endocrine Disruption
VII	Response to Ecosystem Perturbation…Human
VIIA	Response to Ecosystem Perturbation…Ecosystem-Wide

Intellectual Development
None

Useable Environmental Technologies

While no technologies have resulted directly from the e.hormone workshop series, the CBR deems that interdisciplinary workshops like this one are critical for the creation of scientific collaborative approaches that foster biosensor development. Such biosensors harness the power of "environmental signaling," and lead us to further exploration and development of numerous near real-time monitoring technologies for the ONR, in particular, and the DOD, in general.

Effects of Estrogens and Endocrine disrupters on Suppression of Apoptosis in normal and neoplastic Breast epithelial cells

Principal Investigator: Matthew E. Burow, Ph.D.
Assistant Professor
Center for Bioenvironmental Research
Department of Medicine and Surgery
Tulane University Health Science Center

Co-Investigator: John A. McLachlan, Ph.D.
Weatherhead Distiguished Professor and Director
Center for Bioenvironmental Research and
Department of Pharmacology
Tulane University Health Science Center

Reporting Period: August 2002 – July 2003

Primary Objectives of Research Activities

The major goals of this project where to 1) develop technologies and methods to identify relevant organochlorine chemicals and dietary flavonoids that exert effects on estrogen responsive tissues and cell survival pathways, and 2) identify mechanism by which selected environmental agents would subvert the estrogen and cell survival signaling pathways thereby leading to potential developmental defects and or disease states (i.e. cancer).

Progress Made to Achieve these Objectives

During the funded period we have identified a role for specific signaling pathways including the mitogen-activated protein kinase pathway (MAPK) functioning through AP-1 mediated transcription as a critical component of the estrogen mediated cell survival signaling pathway. The ability of estrogenic chemicals (estradiol, DES, DDT) to exert effects on cell survival pathways of breast carcinoma cells required an intact ERK-MAPK-pathway (1,4). In contrast anti-estrogens such as tamoxifen or ICI 182,780 as well as dietary flavonoid anti-estrogens exert negative effects on cell survival and ER activity through the JNK and p38 pathways (2-4,7). Interestingly our recently submitted findings suggest that the DDTs can also function to induce apoptotic pathways at higher doses (10).

The understanding of the basic mechanisms of cell survival signaling through ER, AP-1 and MAPKs allowed us to develop in vivo screening technologies for AP-1 activating chemicals using stably transfected human endometrial and human embryonic kidney cell lines. These cell systems have allowed us to examine the ability of selected chemicals to activate AP-1 and related signaling pathway (Fos, Jun, Creb, Elk, Chop) through ER–dependent and independent mechanisms (5,6). The ability of environmental agents to regulate cell signaling and AP-1 pathways in an ER-independent mechanism forces us to expand our search for relevant endocrine disruption chemicals to include those outside the realm of estrogenic compounds. Subsequent to this an additional manuscript has been completed during the 2002-2003 year. This publication (9) now demonstrates the ability of specific organochlorine chemicals to signal to AP-1 mediated gene expression via

activation of selective MAPK pathways. In this publication we demonstrate a primary role for the p38-MAPK cascade but not Erk, JNK or BMK-1 in regulation of AP-1 by organochlorines. Subsequent studies have now established that specific organ chlorines can activate a number of other signaling pathways including antioxidant response elements, estrogen response elements (ERE), hypoxia-induced factor (HIF) and cyclic AMP response element (CRE). These finding have been submitted for publication (12, 13). This suggests that common targets exist for the organochlorine/p38-MAPK cascade in the regulation of environmental responsive gene expression. The ability of organ chlorine to activate MAPK and potentially mediated cell survival/death signaling in human tissues is supported by our in press work demonstrating that activation of p38 signaling can promote cell survival and therapeutic resistance in breast carcinoma cells (11). We have also identified that the transcriptional coactivator CBP and P300 represent targets of organochlorine/p38-MAPK mediated phosphorylation. The ability of CBP/p300 to control transcriptional activation by a number of transcription factors (CREB, AP-1, HIF ER etc.) further strengthens our hypothesis that environmental signaling to gene expression occurs through a common transcriptional target (13). We are now focusing our efforts on identifying the mechanisms and role of organ chlorine MAPK targeting of coactivators.

Major Accomplishments

- Established role for specific MAPKs (p38) in organ chlorine regulation of AP-1-mediated gene expression (publication #9)
- Identified a role for MAPK signaling in conjunction with Bcl-2 expression in estrogen mediated cell survival signaling in breast carcinoma cells. (publication # 4)
- Used relevant estrogen responsive reporter technologies to screen flavonoid phytochemicals for estrogenic and anti-estrogenic activities towards MCF-7 breast carcinoma cells (publications #2,3)
- Identified a role for JNK and p38 MAPKs in signaling by flavonoid phytochemicals in the regulation of ER-mediated gene expression and proliferation of breast carcinoma cells (Publication # 2,5,7)
- Identifies those flavonoid phytochemicals that demonstrate the ability to induce apoptosis or programmed cell death in human breast carcinoma cells (publication #8)
- Correlated relative estrogen receptor alpha and beta expression and signaling with apoptotic sensitivity and resistant among breast cancer cell variants (manuscript #1)
- Developed an *in vivo* mammalian cell culture assay to examine environmental relevant organ chlorine molecules for estrogen receptor dependent and ER-independent activity toward cell signaling via mitogen-activated protein kinase (MAPK)-mediated activation protein 1 (AP-1) transcription. (publications # 4,5)
- Identified a role for organ chlorine pesticides and flavonoid phytochemicals signaling to AP-1 via ER independent mechanisms (publications #5-7, 9).

Publications, Manuscripts, Abstracts

Burow, M.E., Weldon, C.B., Chiang, T-C., Tang, Y., **Collins-Burow B.M.,** Rolfe, K., Li, S., **McLachlan, J.A.,** Beckman, B.S. Differences in protein kinase C and estrogen receptor α, β expression and signaling correlate with apoptotic sensitivity of MCF-7 breast cancer cell variants. *Int. J. Oncol.* **16**: 1179-1187, (2000).

Collins-Burow, B.M., Burow, M.E., Duong, B.N., **McLachlan, J.A.** Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms. *Nutrition and Cancer.* **38(2),** 229-244 (2000).

Burow, M.E., Boue, S.B., **Collins-Burow, B.M.,** Melnik, L.I., Duong, B.N., Li, S.F., Wiese, T., Cleavland, E., **McLachlan J.A.** Phytochemical glyceollins, isolated from soy, mediate anti-hormonal effects through estrogen receptor alpha and beta. *J. Clin. Endocrinol. and Metabolism* **86(4)**, 1750-1758, (2001).

Burow, M.E., Weldon, C.B., Tang Y., **McLachlan, J.A.,** Beckman, B.S. Oestrogen -mediated suppression of TNF-induced apoptosis in MCF-7 cells: subversion of Bcl-2 by anti-oestrogens. *J. Steroid Biochem. & Mol. Biol.* **78(5)**: 409-418, (2001).

Frigo, D.E., Duong, B.N., Melnik, L.I., Schief, L., Collins-Burow, B.M., Pace, D.K., **McLachlan, J.A., Burow, M.E.** Flavonoid Phytochemicals Regulate Activator Protein-1 Signal Transduction Pathways in Endometrial and Kidney Stable Cell Lines. *Journal of Nutrition* **132(7)**:1848-1853, (2002).

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T-C., **McLachlan, J.A.** DDT and its metabolites alter gene expression in human uterine cell lines through ER-independent mechanisms. *Environmental Health Perspectives* **110(12)**:1239-1245, (2002).

Burow, M.E., Collins-Burow, B.M., Frigo, D.E., Weldon, C.B., Elliot, S., Alam, J., **McLachlan, J.A.** Antiestrogenic activity of flavonoid phytochemicals mediated via c-jun N-terminal protein kinase and p38, Mitogen-activated protein kinase pathways. Isoform specific antagonism of estrogen receptor alpha. Submitted to *Endocrinology*. (2004).

Collins-Burow, B.M., Burow, M.E., Weldon, C.B., **McLachlan, J.A.** Induction of apoptosis by anti-estrogenic phytochemicals in breast carcinoma cells. Manuscript in preparation for submission.

Frigo, D.E., Tang, Y., Beckman, B.S., Scandurro, A.B., Alam, J., **Burow, M.E., McLachlan, J.A.** Mechanism of AP-1-mediated gene expression by select organ chlorines through the p38 MAPK pathway. *Carcinogenesis* 25(2): 249-261, (2004).

Frigo, D.E., Vigh, K.A., Struckhoff, A.P., Elliott, S., Beckmans, B.S., **Burow, M.E., McLachlan, J.A.** Xenobiotic-induced TNF expression and apoptosis through the p38 MAPK signaling pathway. Submitted to *Toxicology Letters* (2003).

Weldon, C.B., Parker, A.P. Patten, D., Elliot S., Tang, Y., **Frigo, D.E.,** Dugan, C.M., Coakley, E.L., Butler, N.N., Clayton, J.L., Alam, J., Curiel, T.J., Beckman, B.S., Jaffe, B.M., **Burow, M.E.** Sensitization of Apoptotically-Resistant Breast Carcinoma Cells to TNF and TRAIL by Inhibition of P38 Mitogen-Activated Protein Kinase Signaling. *International Journal of Oncology* 24: 0-00, (2004). In press

Frigo, D.E., Simpson E.N., Weldon, C.B., Elliott, S., Melnik, L.I., Dugan, C.B., Collins-Burow, B.M., Zhu, Y., Salvo, V.A., Lopez, G.N., Kushner, P.J., Curiel, T.J., **McLachlan, J.A., Burow, M.E.** The p38 MAPK Stimulates Estrogen-Mediated Transcription and Proliferation Through the Phosphorylation and Potentiation of the p160 Coactivator GRIP1. Submitted to *Mol. Endocrinology* (2004).

Frigo, D.E., Vigh, K.A., Burow, M.E., McLachlan, J.A. Phosphorylation and Potentiation of the transcriptional coactivators p300 and CBP by the p38 MAPK Submitted to *J. Biol. Chem.* (2004)

Presentations

Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., **McLachlan, J.A**. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. AACR Annual Meeting. March 28th, 2001-New Orleans, LA.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 13th annual Tulane Health Sciences Research Days, 2001.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2001 Environmental Signals and Sensors Center for Disease Control Meeting.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. Gordon Research Conference, Hormonal Carcinogenesis. July 10th, 2001-Meriden, NH

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T.C., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: Mechanisms of Environmental Signaling. **E.Hormone 2001 Conference**. At Tulane University, Oct. 18th, 2001-New Orleans, LA.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T.C., **McLachlan, J.A.**The effects of DDT and its metabolites on AP-1 activity: mechanisms of environmental signaling (Abstract 5126). Proceedings of the American Association for Cancer Research, 2002

Daniel E. Frigo, Matthew E. Burow, Jawed Alam, and **John A. McLachlan.** Endocrine Disruptors Potentiate Coactivators: The role of MAPKs. **E.Hormone 2002 Conference**, at Tulane University. Oct. 17th, 2002-New Orleans, LA

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T-C., Alam, J., **McLachlan, J.A.** Endocrine disruptors potentate coactivators: The role of MAPKs. 14th Annual Tulane Health Sciences Research Days, 2002.

Burow, M.E., Melnik, L.I., Elliot, S.E., Collins-Burow, B.M., Alam, J., Hill, S.M., Beckman, B.S., **McLachlan, J.A.** G-Protein Regulation of Nuclear/Steroid Hormone Receptor Activation Through p160 Coactivator Targeting. Keystone Symposia, Nuclear Receptor Superfamily 2002.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T-C., **McLachlan, J.A.** Endocrine disruptors potentiate coactivators: The role of MAPKs. Keystone Symposia, Nuclear Receptor Superfamily 2002.

Intellectual Development

1. **Student(s) name**: Frigo, Daniel, Vigh, Katinka, and Simpson, Erica

2. **Period of funding**: Frigo, Daniel E. (Graduate Student 2002-2003), Katinka Vigh (Undergrad Thesis project 2002), Erica Simpson (Graduate Student 2002-2003)

3. **Duties and Responsibilities**:

   (Frigo - Ph.D. completed December 2003)
   Dissertation title: "DDT AND ITS METABOLITES SIGNAL NUCLEAR TRANSCRIPTIONAL REGULATORS THROUGH A NON-ER-MEDIATED MECHANISM: A MODEL OF ENVIRONMENTAL STRESS SIGNALING"
   Present: Post-doctoral fellow Duke University

   Vigh - Tulane University (B.A. received 2002)
   Present: Graduate Student University of Chicago

   Erica Simpson is a 3rd year Graduate Student in the laboratory of John A. McLachlan that has been continuing some of the research on organ chlorine signaling initiated by Daniel Frigo.

Useable Technologies

During the funding period in vivo cell culture models have been established for the examination of cell signaling pathways activation by relevant environmental contaminants. The cell systems can be utilized to screen extracts, mixtures of individual chemicals for activity on known cellular events involved in environmental toxicant responses. This type of screening would provide information as to potential deleterious effects of certain environmental chemicals or methodologies to classify these chemicals based upon unique cell-signaling profiles.

Characterization of Novel Antibodies for Autonomous Underwater Vehicles

Principal Investigator: Robert Blake II, Ph.D.
Professor and Chair,
Division of Basic Pharmaceutical Sciences,
Xavier University

Co-Investigators: Diane A. Blake, Ph.D.
Professor
Department of Biochemistry
Tulane Health Sciences Center

Reporting Period: August 2002 - July 2003

Primary Objectives of Research Project

The long-term objective of this research is to develop biosensors that will permit the rapid automated identification and quantification of environmental contaminants. A set of high affinity, highly selective binding reagents (antibodies) is envisioned that will permit the development of portable immunosensors that can rapidly and accurately quantify environmental antigens of interest in real time in the field. The immediate objectives of the progress summarized herein was to conduct detailed studies on the binding properties of two novel antibodies (designated as 5B2 and 2D42) that are destined to be incorporated into an Autonomous Underwater Vehicle (AUV) as part of our ongoing developmental activities funded by the Office of Naval Research. Both antibodies exhibit unexpected binding properties that must be characterized and understood before either protein is incorporated into the AUV. It is anticipated that these binding studies will also provide fundamental information on the mechanism(s) of the unexpected binding synergy observed with these antibodies.

Progress Made to Achieve these Objectives

Antibody 5B2 exhibits a wide range of allosteric behaviors.

Detailed equilibrium binding studies were conducted on a monoclonal antibody directed against Pb(II) complexed with a protein conjugate of DTPA (diethylenetriamine-N,N,N',-N'',N''-pentaacetic acid). This antibody, designated as 5B2, exhibited a remarkable range of allosteric binding reactions when the antibody was presented with selected chelators, metal-chelator complexes, or various combinations of the two. Under appropriate solution conditions, this antibody exhibited:

(i) Normal homogeneous binding – binding curves obtained with DTPA or a cyclohexyl derivative of DTPA in the presence and absence of numerous metal ions were consistent with the anticipated one-site homogeneous binding model;

(ii) Homotropic positive cooperativity – binding curves obtained with aminobenzyl-DTPA or its complexes with Ca(II), Sr(II), and Ba(II) indicated that the apparent affinity of the antibody

increased as the concentration of chelator-metal complex increased. The resulting highly sigmoidal binding curves were characterized by Hill coefficients of 2.3 to 6.5;

(iii) Heterotropic positive cooperativity – although individual binding curves obtained with the Pb(II) and In(III) complexes of aminobenzyl-DTPA were hyperbolic, in both cases the apparent affinity of the antibody for the chelator-metal complex was higher in the presence of excess chelator than it was in the presence of excess metal ion;

(iv) Homotropic negative cooperativity – binding curves obtained with the aminobenzyl-DTPA complexes of Hg(II) or Cd(II) indicated that the apparent affinity of the antibody decreased as the concentration of the chelator-metal complex increased; and

(v) Heterotropic negative cooperativity – further binding studies using carefully defined mixtures of chelator, Hg(II), and Cd(II) demonstrated that the aminobenzyl-DTPA-Hg(II) complex opposed the binding of additional chelator-metal complexes more strongly than did the aminobenzyl-DTPA-Cd(II) complex.

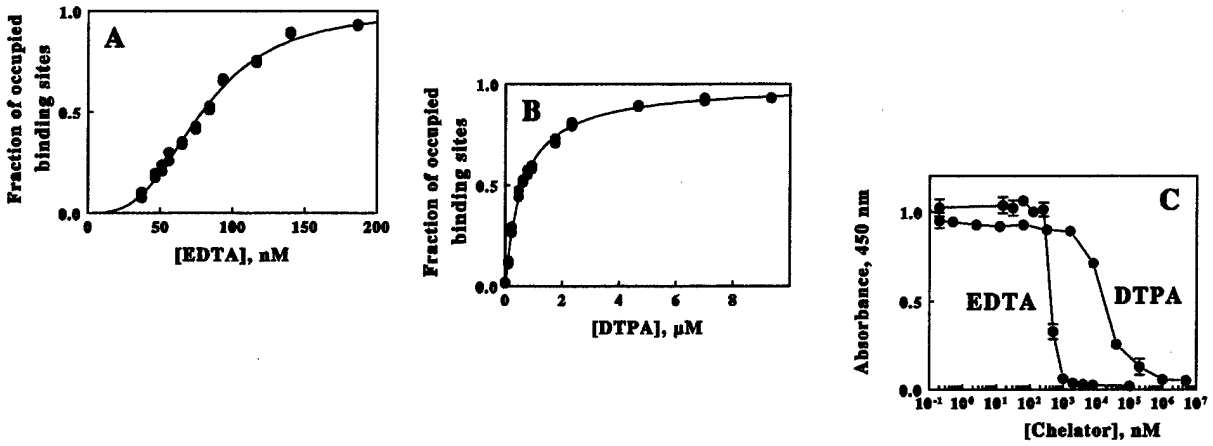
The same range and degree of allosteric binding behavior was also observed with the corresponding Fab fragments prepared by proteolytic cleavage of the intact 5B2 antibody. The Fab fragment differed from the intact antibody only in that the apparent affinity of the Fab was generally lower than that of the parent antibody for any given chelator-metal complex.

One hypothesis to account for this behavior is that the highly charged chelator-metal complex binds to both the traditional antigen binding sites and to multiple charged sites on the surface of the Fab portions of the intact antibody, and that antigen occupancy at these nontraditional, 'extra' sites is associated with conformation changes that influence the affinity of the traditional antigen binding site.

Other antibodies, such as 2D42, bind their antigen with positive cooperativity that is characterized by a Hill coefficient of greater than two.

In addition to antibody 5B2, our laboratories have partially characterized the functional properties of three other monoclonal antibodies directed against metal-free chelators (2D42, 1B11, and 15B4) that yield equilibrium binding curves with novel features. Binding experiments conducted on the KinExA with antibody 2D42 and selected metal-free chelators are presented in Figs. 1 and 2. Fig. 1A shows the sigmoidal binding curve obtained when purified 2D42 was mixed with different concentrations of EDTA (ethylenediamine-N,N,N',N'-tetraacetic acid). The best fit of the sigmoidal curve to the data was obtained with a Hill coefficient of 3.1. Fig. 1B shows the 'normal' hyperbolic binding curve obtained when 2D42 was mixed with different concentrations of DTPA, which differs structurally from EDTA by one acetic acid and one ethylene amine group. DTPA bound to the antibody with lower affinity than did EDTA and showed no evidence of positive cooperativity. Fig. 1C shows a comparison of the binding of EDTA and DTPA to 2D42 using a standard ELISA assay. The concentration of DTPA required to competitively inhibit between 10 and 90% of the binding of soluble 2D42 to the antigen immobilized within the ELISA plates covered a span of approximately 1.8 log units, the 'normal' range predicted by the one-site homogeneous binding model. Conversely, the concentrations of EDTA required to competitively inhibit binding of 2D42 to the same immobilized antigen

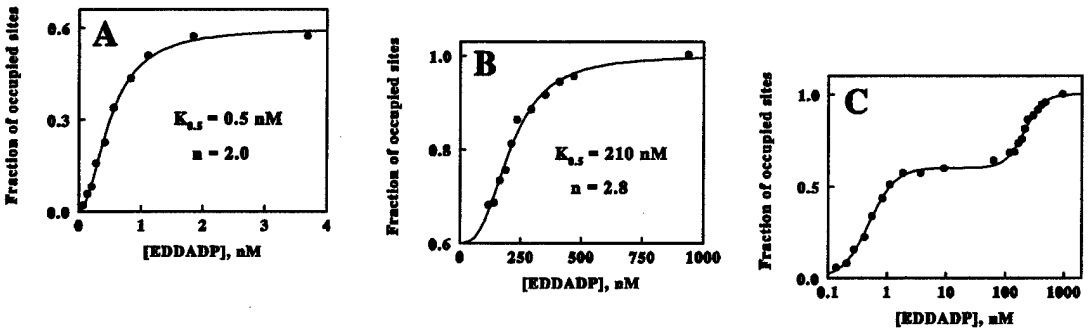
Figure 1. Binding of EDTA and DTPA to 2D42 by KinExA and ELISA.



covered a much smaller span of less than 1.0 log units, an observation consistent with the highly cooperative binding behavior observed using the KinExA. Thus, the results obtained by ELISA were qualitatively identical to those obtained using the KinExA. Although this favorable comparison provided evidence that the novel results obtained on the KinExA were due solely to the novel binding properties of the antibodies themselves, there is general agreement that binding data generated by competitive ELISA are qualitative at best.

The data presented in Fig. 2 summarize the highly unusual results obtained in KinExA experiments when 2D42 was mixed with EDDADP (ethylenediamine-N,N-diacetic-N',N'-dipropionic acid), which differs structurally from EDTA by only two methylene carbons. Fig. 2A shows the sigmoidal concentration dependence obtained at low EDDADP concentrations for occupancy of roughly 60% of the available antigen binding sites in the entire preparation of purified antibody molecules. Fig. 2B shows the sigmoidal concentration dependence obtained at high EDDADP concentrations for the occupancy of the remaining 40% of the antigen binding sites in the population. Fig. 2C shows a semilog plot of the same data that simply combines and summarizes in one panel these highly unusual results. This is the same antibody preparation that yielded the hyperbolic binding curve with DTPA featured above in Fig. 1B. The working hypothesis is that these odd results obtained with 2D42 are simply further manifestations of the hypothesis advanced above using antibody 5B2. That is, that the highly charged EDDADP binds to both the traditional antigen binding sites and to multiple charged sites on the surface of the antibody, and that antigen occupancy at these nontraditional, 'extra' sites is associated with conformation changes that influence the affinity of the traditional antigen binding site.

Figure 2. Binding of EDDADP to antibody 2D42



Major Accomplishments

- These are the first detailed descriptions of allosteric binding behavior by antibodies. The observations summarized in this report provide new insights into a fundamental property of antibody functional behavior that appears to have been largely unnoticed.

Publications

Blake, II, R.C., Delehanty, J.B., Khosraviani, M., Yu, H., Jones, R.M., and Blake, D.A. (2003) "Allosteric Binding Properties of a Monoclonal Antibody and Its Fab Fragment", *Biochemistry* **42**, 497-508

Presentations

Blake, II, R.C. "Synergy in antibody-antigen binding interactions", invited seminar at the University of the Virgin Islands, St. Thomas, VI, September 13, 2002

Blake, II, R.C., Jones, R.M., Lackie, S., and Blake, D.A. (2003) "In-line Uranium Immunosensor", DoE-NABIR PI Workshop, Warrenton, VA, March 19

Blake, II, R.C "Allosteric binding in antibodies and protein antigens", annual ARCH Research Symposium, New Orleans, LA, April 21. 2003

Intellectual Development

1. **Student(s) name**: Johnson, Renee, Borders, Wayne and Bolden, Tiffany
2. **Period of funding**: Ms. Renee Johnson, for fall, 2002; Mr. Wayne Borders, for fall and spring; Ms. Tiffany Bolden, for fall and spring; and Ms. Teresa Jackson, for fall and spring.

3. **Duties and Responsibilities**: Each student participated in the instrumental analysis of the equilibrium binding studies using the KinExA flow fluorimeter.

Useable Environmental Technologies

As stated above, the immediate objectives of the Xavier portion of this overall project were to conduct detailed fundamental studies on the binding properties of two novel antibodies (designated as 5B2 and 2D42) that are destined to be incorporated into the Autonomous Underwater Vehicle (AUV) as part of our ongoing developmental activities. The approximate division of work is that the fundamental kinetic and thermodynamic studies are to be conducted at Xavier, while the more applied developmental studies are to be conducted by Dr. Diane Blake at Tulane.

L1 Retrotransposition: A Biomarker for Exposure to Low-levels of Environmental Pollutants

Principal Investigator: Shubha Kale Ireland, Ph.D.
Associate Professor
Department of Biology
Xavier University of Louisiana

Reporting Period: August 2002 – July 2003

Primary Objectives of the Research Project

To develop and optimize accurate cell culture assays to measure L1 retrotransposition in mammalian (mouse and/or humans cells).

To test the effects of low-level exposures of certain commonly found environmental pollutants (example heavy metals) on the frequencies of L1 retrotranspositions.

To establish increased L1 retrotransposition as an effective biomarker of exposure to genotoxic chemicals in nature.

Progress Made to Achieve these Objectives

1) Development and optimization of a new assay to measure L1 insertions

Rationale: While the L1 assay optimized in our laboratory (in conjunction with Dr. Prescott Deininger of Tulane University) is very accurate, it is extremely labor and time consuming. In fact, the sheer time factor involved (it takes a minimum of 7-8 weeks to complete one part of the assay, another 7-8 weeks to do the second part) increases the chances of contamination. Therefore we sought to develop a transient assay for L1 frequency measurement, which is equally effective but is relatively short (completed in about 4 weeks). Another advantage of the transient assay is that it uses human (HeLa) cells as opposed to the original assay, which uses mouse fibroblast (3T3) cells. Finally, having two assays gives a better opportunity for comparing the results obtained, specifically with respect to the effects of the heavy metals in mouse versus the human cells.

2) Results of experiments using the new L1 transient assay using filtered HgS

HgS is a known carcinogen (especially because of its low solubility in water). The concentrations tested ranged between 0.5 to 27.4 ppb [using 1microgram each of the L1 and the pires (control) DNA]. For this chemical, positive results were obtained with the 4.6 ppb showing the maximum increase in the L1 retrotransposition of the value of 2.6-3 fold (compared to the controls).

While these increases may seem modest, in biological systems, they are significant, especially since clinical research has already linked increased L1 jumping to human diseases including certain types of cancers.

Major Accomplishments

- Developed and optimized a new assay (transient assay) to accurately measure L1 Retrotransposition. This work was done in collaboration with Dr. Prescott Deininger (CBR) of Tulane University.

Publications, Manuscripts and Abstracts

Harris, K., Nguyen, T. Q., Nguyen, Q., Brumfield, K., Kwang, S. and **Ireland, S. K.** Comparison of Genotoxic Effects of Particulate Versus Filtered Cadmium sulfide on Human Retrotransposition. (2002) abstract

Ireland, S. K., Collaborative Workshop in Biomedical Research among Research Centers in Minority Institutions 2003 Spring Symposium (April 28 -29 2003). Funded by the NIH (National Institutes of Health) abstract

Presentations

Same as above (this was an oral, power-point presentation).

Intellectual Development

1. **Students Trained**: Kelley Harris, Kristi Blumfield, Sarah Kwang

2. **Period of Funding**: (2002-2003)

3. **Brief Description of Duties and Responsibilities**:

   All students participated in every aspect of the research, after going through a rigorous training session in safety. They assisted in ordering supplies, conducting experiments and analyzing the results. Each one had to also make a presentation at one of the bi-weekly lab meetings.

Useable Environmental Technologies
None

Identification and Characterization of Antibiotic Resistant Riverine Gram Negative Bacteria

Principal Investigator: Tanya K. McKinney, Ph.D.
Assistant Professor
Department of Biology
Xavier University and
Department of Environmental Health Science
Tulane University

Reporting Period: August 2002 – July 2003

Primary Objectives of Research Project

The primary objectives of this study are (1) to isolate and identify antibiotic resistant gram negative bacteria from various sites along the Mississippi River; (2) to phenotypically characterize isolates through the use of biochemical and enzymatic assays; (3) to assess antibiotic resistance pattern of isolates; (4) to genotypically characterize such isolates through the use of pulse field gel electrophoresis.

Progress Made to Achieve These Objectives

Four sample sites located within the city of New Orleans, LA were randomly selected. Site 1 is located downstream of a water treatment plant upstream from the city zoo. Site 2 is located at a ferry landing on the east bank of the river. Site 3 is located in area with a high degree of tourist traffic. Site 4 is located at the ferry site located on the west bank of the river. Approximately 230 gram negative isolates have been isolated from Site 1, 109 from Site 2, 183 from Site 3, and 89 from Site 4. Of these only 103 have been identified. Some strains were unculturable following initial isolation. Biochemical analysis using the BBL Crystal Identification system and indole and oxidase testing indicated that the major genera of these bacteria were *Aeromonas, Chromobacterium,* and *Pseudomonas.* The majority of the isolated organisms were resistance to ampicillin and at least one other antibiotic (streptomycin, erythromycin, tetracycline, ciprofloxacin, or Bactrim).

To determine if resistance was intrinsic or extrinsic in nature, plasmid analysis was conducted. However, of the 23 *Aeromonas hydrophilia* strains studied, only one had an observable plasmid.

Presentations

Cristal Webb and Victor Njoku, **Isolation and Identification of Gram Negative Riverine Bacteria.** Undergraduate Science and Engineering Research Conference, Tuskegee University, November, 2003.

Intellectual Development

1. Student(s) Name:

Ms. Candice Williams
Ms. Cristal Webb
Mr. Victor Njoku

2. Period of Funding

Ms Williams – January 2003-May 2003
Ms Webb and Mr. Njoku – May 2003-July 2003

3. Brief Description of duties and responsibilities

Each student was responsible for obtaining and processing samples from each site. Students plated, isolated, gram stained, characterized, and identified bacteria species.

Useable Environmental Technologies
None

Human Health Applications

Principal Investigator: Valerie Wilson, Ph.D. *formerly*
Clinical Associate Professor, Environmental Health Sciences
and Deputy Director
Center for Bioenvironmental Research
at Tulane and Xavier Universities

Reporting Period: August 2002 – July 2003

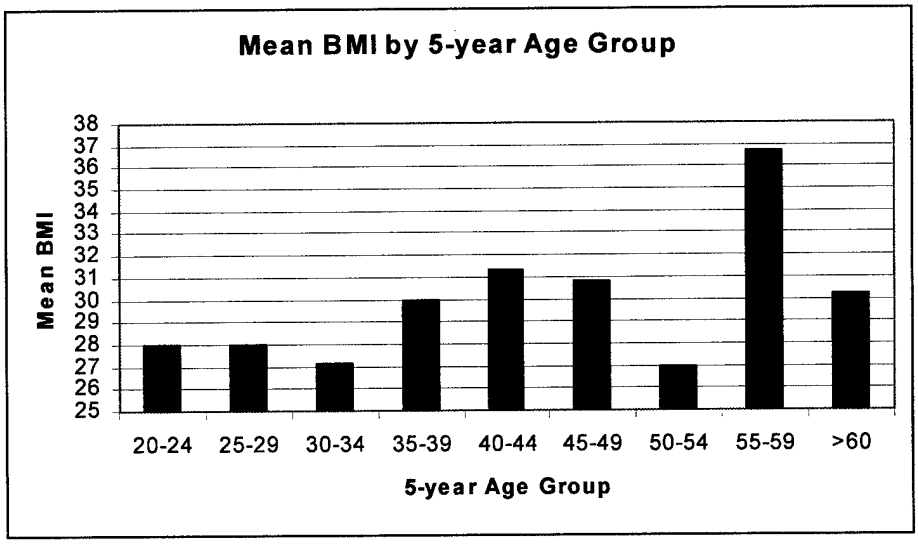
Primary Objectives of Research Activities

- Study genetic and biologic markers related to regulation of uterine function in women
- Study metabolic and hormonal differences
- Evaluate role of biologic and environmental factors

Progress Made to Achieve these Objectives

Since summer 2000, we have continued chart abstraction of material from Medical Center of Louisiana at New Orleans (also known as Charity Hospital) and a private physician's office. We abstracted demographic information, symptomatology, and fibroid pathology from an additional 243 subjects that underwent treatment for uterine fibroids from July 1995 to June 2000, for a total of 426 subjects. We have tabulated and analyzed the broader case distributions, geographic locations, as well as other basic information that characterize the populations. We also performed two preliminary analyses of the database. Each study focused on the different medical treatment that each study population received. The first study analyzed the entire cohort, including hysterectomy and myomectomy cases. The second study examined the hysterectomy cases only.

Major Accomplishments

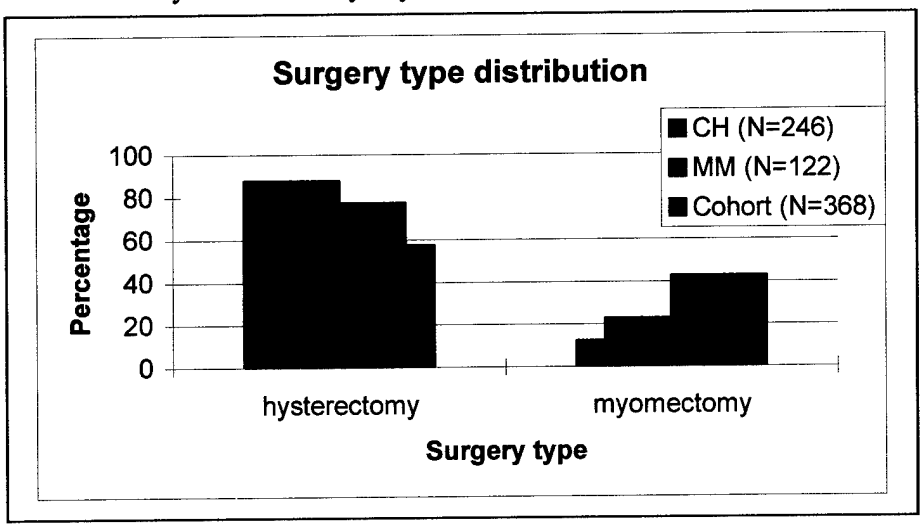


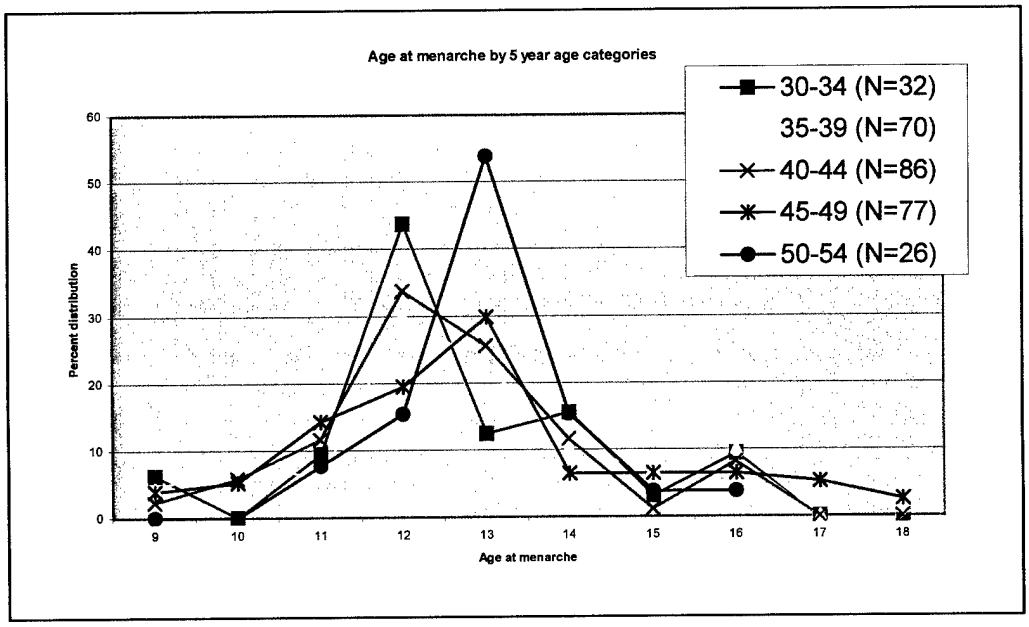
- The purpose of Study I was to provide a description of the potential risk factors for uterine fibroids. Surgical patient charts of women who underwent treatment for uterine fibroids between July 1995 to June 2000 at Charity Hospital and a private physician's office in Metairie were analyzed. Records were utilized that met inclusion criteria which resulted in analysis of demographic information, reproductive

history, symptomatology, and fibroid and uterine pathology from 368 patients. Of the 426 potential patients, 368 had sufficient information to be included in analysis. We analyzed these subjects based on age, race, BMI, age at menarche, and age at menarche by 5-year age category.

Results: The enhanced analyses are virtually identical to the results we described. Previously most patients were African-American women (80%), between the ages of 30 and 44 with average age at menarche between 12 and 13 years. Most women (42%) were classified as obese (BMI > 30). Most women over 35 years (with the 50-54 year age group as the exception) had a BMI greater than 30, or a classification as "obese." In the figure above, levels over 30 indicate a mean BMI over the "obese" standard.

Of the women in both cohorts, 75% had undergone hysterectomy as treatment.
Prior analysis of age at menarche by age decile (10 year blocks) revealed that women in their 30's had a younger mean age at menarche than women in their 40's. With a larger data set, we were able to stratify the results by 5-year cohorts to see if there were any abrupt transitions in the shift of age of menarche. Our data indicate that a major shift in age of menarche occurred in those women 44 years of age or younger, as compared to women 45 years or older. This supports literature findings, and may also indicate an environmental change or some unknown prevalent in the late 50's or early 60's.





- The purpose of Study II was to evaluate if any of the above factors might be correlated to increased fibroid number among women who underwent hysterectomy only. A retrospective cross-sectional chart review was conducted of women in the Greater New Orleans area who presented at one of two clinical settings seeking definitive treatment for uterine fibroids. Selection criteria for this study were: age between 25-64; pre-menopausal stage; uterine fibroids as the primary diagnosis; hysterectomy between July 1995 and June 2000; and residency in the Greater New Orleans area, as determined by zip code. Demographic information, symptomology, and fibroid pathology data were abstracted. Of the potential patients at both clinics, only 155 had sufficient information in their charts and met the inclusion criteria.

Results: After careful review of the chart information, none of the risk factors examined in Study 1 were significant predictors of multiple uterine fibroids, though BMI (a measure of obesity) was nearly significant. This study was inconclusive due in part to inability to obtain a significant number of study subjects and insufficient information in Charity Hospital medical charts. Equally important is the lack of precision in which the information about fibroids is presented. This occurs because there are three principal methods used to estimate fibroid mass: "guesstimates" of the number of fibroids, size comparison to pregnant uteri, and weight estimates. Therefore, unless there is a more uniform methodology applied to fibroid measurement as part of the surgical procedure, hysterectomy data from Charity appears not to be the data source of choice to analyze risk factors among women with many fibroids. Future studies that examine fibroid number should use either prospective data or patients who underwent myomectomy, in which case the size and number of the fibroids removed are accurately documented.

- Conclusions from the two Studies: New Orleans women with fibroids have a demographic and health profile similar to that of women in other cities. The decline in the age of menarche may implicate an environmental factor (yet to be defined) in the condition.

Publications

Moorehead, M., and Conard, CJ. Uterine Leiomyoma: A Treatable Condition. 2001. *Annals of the New York Academy of Sciences* 948: 121-129.

Presentations

Moorehead, M. "Uterine Fibroids: A Treatable Disease," e.hormone 2000 Symposium, New Orleans, October 15-18, 2000.

Intellectual Development
None

Useable Technologies
None

Synthesis of Diiron Complexes Containing New Phosphido-bridging Ligands

Principal Investigator: Michael R. Adams, Ph.D.
Associate Professor
Department of Chemistry
Xavier University of Louisiana

Reporting Period: August 2002 – July 2003

Primary Objectives of Research Project

The primary objective of this project is to develop synthetic routes to diiron complexes containing modified phosphido ligands. A secondary objective is, necessarily, to develop syntheses of appropriate phosphine ligands to be used as precursors to the target iron compounds. The goal of this work is to determine if appropriate modification of bridging phosphido ligands in the diiron complexes will significantly increase the solubility of these compounds in environmentally friendly solvents.

Progress Made to Achieve these Objectives

Two undergraduate students were hired as laboratory assistants to conduct work on this project. In addition to carrying out lab work, these students were trained to properly search the literature. Through their library work both students contributed significantly to the development of specific plans for experiments to be conducted in the laboratory.

We chose to focus our efforts on synthesis of new diorganochlorophosphines, PR_2Cl, bearing long perfluorinated organo substituents. A number of potential synthetic routes based on literature reports of syntheses of simpler phosphines were considered. Our initial efforts were aimed at production of such phosphines via reaction of the Grignard $CF_3(CF_2)_6CH_2CH_2MgI$ with PCl_3.[1] After considerable training in inert atmosphere techniques; the student assistants were able to produce the desired Grignard successfully. Subsequent reaction of this Grignard with PCl_3 produced a mixture of products, a result that was not necessarily unexpected. However, we encountered significant difficulty in separation of these products, with considerable decomposition occurring during the purification phase. Further work towards optimizing this procedure was explored, but with little success.

An alternate route to the desired phosphines involved reaction of appropriate Grignards with diethylphosphoramidous dichloride, $(C_2H_5)_2NPCl_2$, followed by treatment with HCl.[2] The most promising result has been achieved via reaction of $CF_3(CF_2)_6CH_2CH_2I$ with Mg to produce the Grignard $CF_3(CF_2)_6CH_2CH_2MgI$. Addition of an excess (3 equivalents) of this Grignard to an ether solution of $(C_2H_5)_2NPCl_2$ produces the desired intermediate, $[CF_3(CF_2)_6CH_2CH_2]_2PN(CH_2CH_3)_2$, as evidenced through ^{31}P and ^{1}H NMR studies. However, spectroscopic data also suggest the presence of $[CF_3(CF_2)_6CH_2CH_2]ClPN(CH_2CH_3)_2$.

Upon treatment of petroleum ether solution of the above product with hydrogen chloride, the desired phosphine, $[CF_3(CF_2)_6CH_2CH_2]_2PCl$, is obtained. As expected, there is evidence to suggest that $[CF_3(CF_2)_6CH_2CH_2]PCl_2$ is also present in the product mixture. Separation of these two products has proven difficult, but methods for accomplishing this are continuing to be

explored. In addition, changes in reaction conditions aimed at optimizing the yield of the desired phosphine and avoiding production of $PRCl_2$ are being explored.

Attempts to produce a similar phosphine via Grignard reaction with $(C_2H_5)_2NPCl_2$ has been carried out with p-$CF_3(CF_2)_7CH_2CH_2C_6H_4Br$. The product mixtures obtained from both the intermediate step and the final step are quite complex, and there is evidence of significant amounts of unreacted starting materials. Separation and purification of the products obtained from these reactions are ongoing.

A third general synthetic route that has been explored involves a metal -halogen exchange reaction as the initial step and avoids the use of a Grignard reagent. It has been previously shown[3] that reaction of p-$C_6F_{13}C_6H_4Br$ with $CH_3(CH_2)_3Li$ produces p-$C_6F_{13}C_6H_4Li$. Thus, a similar process was attempted using p-$CF_3(CF_2)_7CH_2CH_2C_6H_4Br$ as the starting material and reacting the resulting organolithium product with $(C_2H_5)_2NPCl_2$. Subsequent treatment with hydrogen chloride should lead to the desired PR_2Cl product. Attempts to produce the desired organolithium compound have been hampered by the limited solubility of the starting material at the low temperatures necessary for conducting the reaction. Further exploration of this route, with a focus on alternate solvents, is planned.

Both of the student assistants were hired to work part-time on this project during the spring, and full time for ten weeks in the summer. One of the students planned to continue her work throughout the 2003/04 academic years. In addition to conducting the experiments described above, both students were involved in preparing staring materials (e.g., $(C_2H_5)_2NPCl_2$) for these syntheses.

Major Accomplishments

- Trained two students in necessary lab techniques for continuation of project throughout summer 2003 and 2003/04 academic year.
- Successful production of Grignard precursors to desired phosphines.
- Evidence for successful production of $(C_6F_{13}CH_2CH_2)_2PCl$.

Presentation

Bolden, G., Ray, K. , Adams, M. R., " Progress Toward Synthesis of Phosphido-bridged Diiron Complexes Having Perfluorinated Alkyl Substituents on Phosphorus", presented at 226th National Meeting of the American Chemical Society, Sept. 7-11, 2003

Intellectual Development

1. **Student(s) name**:
   Ms. Gevoni Bolden
   Ms. Kamilah Ray

2. **Period of funding**:
   January 2003 - July 2003

3. **Brief description of duties and responsibilities**:

Ms. Ray and Ms. Bolden shared duties equally. They were responsible for library research, learning inert atmosphere and spectroscopic techniques, and carrying out synthetic procedures in the lab. Both were responsible for keeping accurate and current records of their work and proper reporting of their findings. In addition, they assisted in daily upkeep of the laboratory and equipment, overseeing of correct waste disposal methods, and adherence to proper safety guidelines (e.g., maintenance of MSDS files).

Useable Environmental Technologies

It is anticipated that results from this study will eventually contribute to the development of new catalysts for processes that can be carried out in environmentally friendly solvents.

References

1. www.orgsyn.com (Organic Syntheses)

2. a)Issleib, K., et. al., Chem. Ber., 1959, 92, 2681.
   b) Yudina, et. al., Isvest. Akad. Nauk, SSR., Ser. Khim., 1966, 4, 1954.

3. Hope, E., et. al., J. Chem. Soc. Dalton Trans., 2002, 491

River Communication Core

Principal Investigator: Douglas J. Meffert, Ph.D.
Clinical Associate Professor
Deputy Director
Center for Bioenvironmental Research

Co-Investigators: George Rey
President
COTS Technology, LLC

Reporting Period: August 2002 - July 2003

Primary Objectives of Research Project

For the past five years, Tulane University with the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities have led a partnership with academic, private, and public entities on local, regional, state, and national scales, including the Naval Oceanographic Office (NAVOCEANO) at Stennis, MS, to develop an ambitious project – the Tulane RiverSphere facility (formerly known as the National Center for the Mississippi River). As a lead government partner, NAVOCEANO boasts 170 years of ocean survey experience, a strong and creative group of ocean engineers, a leading ocean modeling capability, growing expertise in autonomous vehicles, and one of the largest and fastest supercomputers in the world.

Oriented toward both research and education, the River Center's mandate is to capture a wide range of scientific and cultural aspects of the Mississippi River system and its influence on the Gulf of Mexico region and America in general. In the long-term, the RiverSphere will fulfill this mandate through the creation of research laboratories, classroom space, conference rooms, exhibition space, and a waterfront performance venue. It will be a major simulation and communication venue for scientists, educators, and the general public for its partners on the scientific and cultural issues related to the River and the Gulf of Mexico. The RiverSphere will also publicize its findings through conferences as well as in print, media broadcast, and web media, while it synthesizes and translates these findings into exhibits, festivals, and traveling displays on the river. The CBR received support from the Office of Naval Research (ONR) to provide support for 6 months to aid in the communication effort of this initiative.

Progress Made to Achieve These Objectives

Laboratories dedicated to long-term river, gulf, ocean and water research, and related biomolecular sciences are a core component of the RiverSphere, leveraging upon the successes of the CBR's Integrated Bioenvironmental Hazards Research Program funded by the ONR (Figure 1). The laboratories are being developed to accomplish projects chosen by scientists working with industry, using state-of-the-art research and experimentation technology. Significant regional issues with national relevance are being explored including pollution, climate, river degradation, coastal erosion and subsidence, and water supply. A multi-disciplinary approach has included fields ranging from biology, geology, engineering, public health, archaeology, to anthropology.

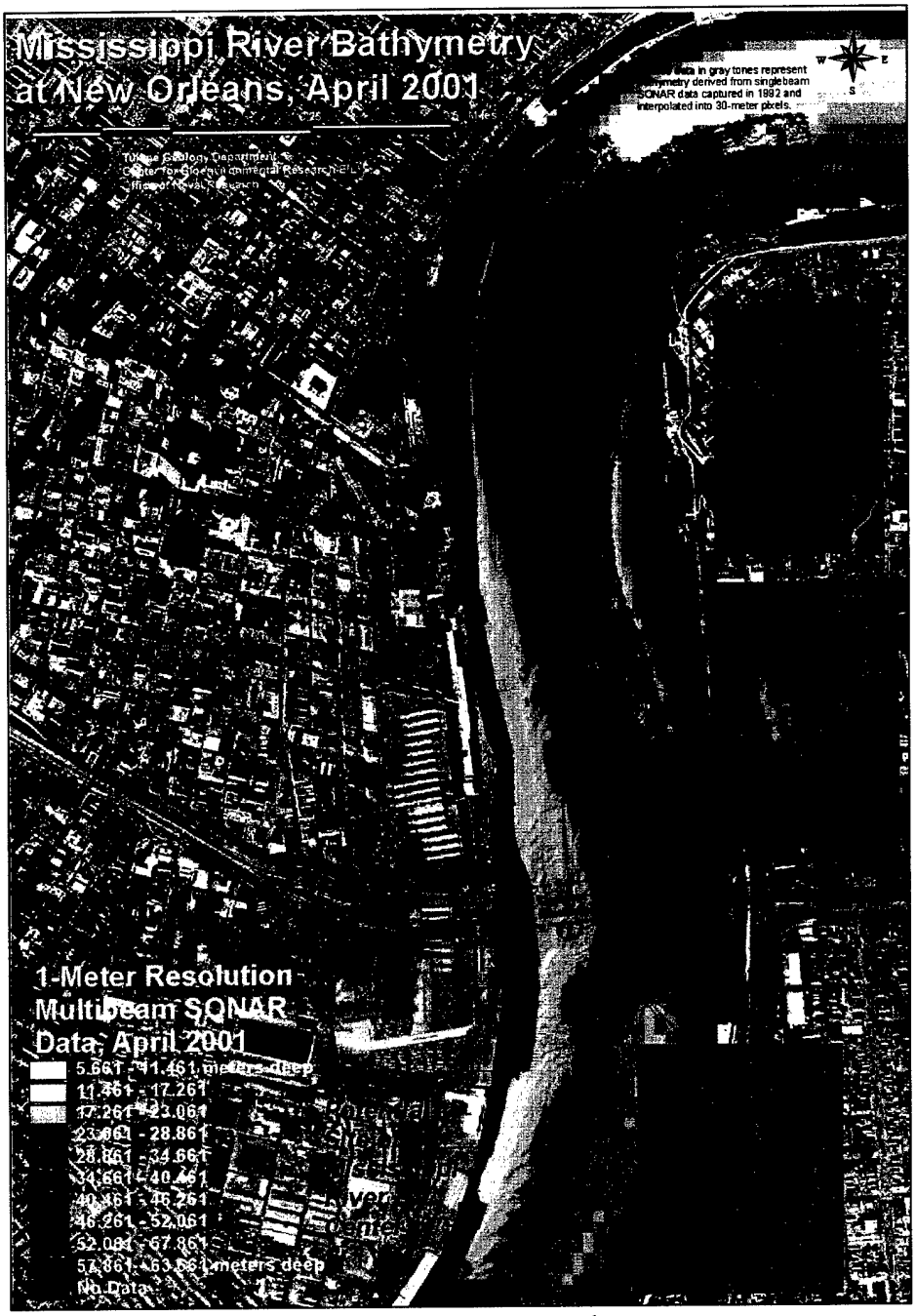


Figure 1. Example of bathymetric research and shipwreck discoveries (obtained through the CBR's Integrated Bioenvironmental Hazards Research Program) in relation to the RiverSphere site.

This collective effort is directed at developing predictive tools that will provide local, regional, and national policy/decision makers with decisive information that can be applied to remedial efforts that will benefit the Mississippi River/Gulf of Mexico estuary system. By utilizing the Mississippi River and the Gulf of Mexico as a natural laboratory, the ONR and CBR have improved the capability of the United States to monitor the risk of exposure to defense related toxicants in systems throughout the world.

Dr. John McLachlan, CBR Director and principal investigator for the CBR's Integrated Bioenvironmental Hazards Research Program is a Co-founding Director for RiverSphere and the principal investigator on this proposal. Working with Dr. McLachlan on this initiative is Dr.

Douglas Meffert, CBR Deputy Director and Project Director for the River Center and Ms. Desiree Johnson, ONR IBHRP and RiverSphere Program Manager. For this proposal, Drs. McLachlan and Meffert utilized funds to support Mr. John M. Barry as a visiting scholar to communicate the River Center partners' environmental and human health research, education, and training programs to diverse public, government, and private audiences. John M. Barry is an internationally renowned and award-winning author of "The Rising Tide: The Great Mississippi River Flood of 1927 and How it Changed America". Mr. Barry brings historical, cultural, and additional scientific expertise to the program and, through his books, has established numerous contacts throughout the country in federal agencies, foundations, private companies, and other individuals that focus on the River, the Gulf of Mexico, and its maritime commerce, navigation, history, and conservation. In addition, funds were utilized to fund Mr. George Rey, President of COTS Technology, LLC to ensure that RiverSphere programming components incorporate the communication needs of ONR and NAVOCEANO, including demonstration projects for remotely operated and autonomous underwater vehicles at the RiverSphere.

Major Accomplishments

- One of the most difficult phases of research on complex, interdisciplinary environmental and health issues is the translation of that basic research into information and technology that can be understood and, more importantly, utilized. For the ONR, this phase involves translating basic science into applications supporting naval operations and educating and informing the public. Effective communication requires both effective venues as well as expertise. ONR support of this initiative has enabled a program for long-term communication of its programs, made available to vast audiences.

Publications (Manuscripts and Abstracts)

Sullivan, M.W., **Meffert, D.J.** and Rey, R.G.: The National Center for the Mississippi River – a Center for Exploring River Culture, Science, and Technology. Proceedings of the MTS/IEEE Oceans 2002 Conference, Biloxi, MI, 4 pp., October 2002.

Presentations

Sullivan, M.W., **Meffert, D.J.** and Rey, R.G: The National Center for the Mississippi River – a Center for Exploring River Culture, Science, and Technology. Oral presentation at the MTS/IEEE Oceans 2002 Conference, Biloxi, MI, October 2002.

Intellectual Development
None

Useable Environmental Technologies
None

Partners (academia, industry, labs/centers, federal agency, etc.)
Naval Oceanographic Office
Tulane and Xavier Universities
COTS Technology, LLC, New Orleans, LA

APPENDIX B.

PUBLICATIONS, MANUSCRIPTS, ABSTRACTS, PRESENTATIONS

Publications, Manuscripts, Abstracts

Publications

Blake, II, R.C., Delehanty, J.B., Khosraviani, M., Yu, H., Jones, R.M., and Blake, D.A. (2003) "Allosteric Binding Properties of a Monoclonal Antibody and Its Fab Fragment", *Biochemistry* **42**, 497-508

Burow, M.E., Boue, S.B., **Collins-Burow, B.M.,** Melnik, L.I., Duong, B.N., Li, S.F., Wiese, T., Cleavland, E., **McLachlan J.A.** Phytochemical glyceollins, isolated from soy, mediate anti-hormonal effects through estrogen receptor alpha and beta. *J. Clin. Endocrinol. and Metabolism* **86(4)**, 1750-1758, (2001).

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Burow, M.E., Weldon, C.B., Tang Y., **McLachlan, J.A.,** Beckman, B.S. Oestrogen - mediated suppression of TNF-induced apoptosis in MCF-7 cells: subversion of Bcl-2 by anti-oestrogens. *J. Steroid Biochem. & Mol. Biol.* **78(5)**: 409-418, (2001).

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Collins-Burow, B.M., Burow, M.E., Weldon, C.B., **McLachlan, J.A.** Induction of apoptosis by anti-estrogenic phytochemicals in breast carcinoma cells. Manuscript in preparation for submission.

Frigo, D.E., Duong, B.N., Melnik, L.I., Schief, L., Collins-Burow, B.M., Pace, D.K., **McLachlan, J.A., Burow, M.E.** Flavonoid Phytochemicals Regulate Activator Protein-1 Signal Transduction Pathways in Endometrial and Kidney Stable Cell Lines. *Journal of Nutrition* **132(7)**:1848-1853, (2002).

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Frigo, D.E., Vigh, K.A., Struckhoff, A.P., Elliott, S., Beckmans, B.S., **Burow, M.E., McLachlan, J.A.** Xenobiotic-induced TNF expression and apoptosis through the p38 MAPK signaling pathway. Submitted to *Toxicology Letters* (2003).

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Harris, K., Nguyen, T. Q., Nguyen, Q., Brumfield, K., Kwang, S. and **Ireland, S. K.** Comparison of Genotoxic Effects of Particulate Versus Filtered Cadmium sulfide on Human Retrotransposition. (2002) - Abstract

Ireland, S. K., Collaborative Workshop in Biomedical Research among Research Centers in Minority Institutions 2003 Spring Symposium (April 28 -29 2003). Funded by the NIH (National Institutes of Health) - Abstract

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Weldon, C.B., Parker, A.P. Patten, D., Elliot S., Tang, Y., **Frigo, D.E.,** Dugan, C.M., Coakley, E.L., Butler, N.N., Clayton, J.L., Alam, J., Curiel, T.J., Beckman, B.S., Jaffe, B.M., **Burow, M.E.** Sensitization of Apoptotically-Resistant Breast Carcinoma Cells to TNF and TRAIL by Inhibition of P38 Mitogen Activated Protein Kinase Signaling. *International Journal of Oncology* 24: 0-00, (2004). In press

Presentations

Blake II, R.C. "Synergy in antibody-antigen binding interactions", invited seminar at the University of the Virgin Islands, St. Thomas, VI, September 13, 2002

Blake II, R.C., Jones, R.M., Lackie, S., and Blake, D.A. (2003) "In-line Uranium Immunosensor", DoE-NABIR PI Workshop, Warrenton, VA, March 19

Blake II, R.C "Allosteric binding in antibodies and protein antigens", annual ARCH Research Symposium, New Orleans, LA, April 21. 2003

Bolden, G., Ray, K. , Adams, M. R., " Progress Toward Synthesis of Phosphido-bridged Diiron Complexes Having Perfluorinated Alkyl Substituents on Phosphorus", presented at 226th National Meeting of the American Chemical Society, Sept. 7-11, 2003

Campanella, R., *Patterns of Bathymetric Change in the Lower Mississippi River, 1893-1992.* Presented in various increments at the Governor's Office of Coastal Affairs' "River Resources" Workshop on February 20, 2003 in New Orleans; Army Corps of Engineers "Comparing Rivers: The Mississippi and the Niger" Conference on November 7-8, 2002, in New Orleans; the Environmental Research Consortium of Louisiana (ERCLA) "Environmental State of the State Conference" Conference, October 11, 2002, in Lafayette; the Louisiana Remote Sensing-Geographic Information Systems Conferences, April 2002, in Baton Rouge. Research co-authored by Dr. Bernard Coakley.

Campanella, R. *Habitat Characteristics of Potential Arbovirus: Field Observations in St. Tammany Parish, Louisiana.* Presented in increments at the Louisiana RS/GIS Conference, April 29-30, 2003 in Lafayette, Louisiana; and Entomological Society of America Conference, November 17-20, 2002, in Fort Lauderdale, Florida.

Burow, M.E., Melnik, L.I., Elliot, S.E., Collins-Burow, B.M., Alam, J., Hill, S.M., Beckman, B.S., **McLachlan, J.A.** G-Protein Regulation of Nuclear/Steroid Hormone Receptor Activation Through p160 Coactivator Targeting. Keystone Symposia, Nuclear Receptor Superfamily 2002.

Frigo, D.E., Burow, M.E., Mitchell, K.A., Elliott, S., **McLachlan, J.A.** The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. AACR Annual Meeting. March 28th, 2001-New Orleans, LA.

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Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T.C., **McLachlan, J.A.** . The effects of DDT and its metabolites on AP-1 activity: mechanisms of environmental signaling (Abstract 5126). Proceedings of the American Association for Cancer Research, 2002

Frigo, Daniel E., Matthew E. Burow, Jawed Alam, and **John A. McLachlan.** Endocrine Disruptors Potentiate Coactivators: The role of MAPKs. **E.Hormone 2002 Conference**, at Tulane University. Oct. 17th, 2002-New Orleans, LA

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Frigo, D.E., Burow, M.E., Mitchell, K.A., Chiang, T-C., **McLachlan, J.A.** Endocrine disruptors potentiate coactivators: The role of MAPKs. Keystone Symposia, Nuclear Receptor Superfamily 2002.

Moorehead, M. "Uterine Fibroids: A Treatable Disease," e.hormone 2000 Symposium, New Orleans, October 15-18, 2000.

Sullivan, M.W., **Meffert, D.J.** and Rey, R.G: The National Center for the Mississippi River – a Center for Exploring River Culture, Science, and Technology. Oral presentation at the MTS/IEEE Oceans 2002 Conference, Biloxi, MI, October 2002.

APPENDIX C.

USEABLE TECHNOLOGIES

Summary of Useable Technologies

Environmental Signals and Sensors

Blake, R.

One technology that is part of the Environmental Signals and Sensors area is:

- Immunosensor for AUV Deployment. This antibody-based biosensor will automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy. A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters. An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has been established. Transfer of the assay to the immunosensor will begin when Sapidyne has corrected the defects in the optical components of the instrument. Sapidyne Instruments (Boise, ID) is constructing the immunosensor and the Tulane laboratory, in conjunction with the Naval Research Laboratory (Dr. Fran Ligler, Washington, DC), is working closely with them to coordinate the development of biological reagents with the development of the instrument. A provisional patent application entitled "Recombinant antibodies that bind to metal-chelate complexes" was filed in March 2001.

Ecosystem Monitoring and Assessment

Meffert, D.

The one technology that is part of the Ecosystem Monitoring and Assessment area is:

- Integrated Autonomous Immunosensor & Autonomous Underwater Vehicle (AUV) System. This system will enhance real-time biosensor deployment for environmental compliance and ultimately biologic warfare detection. The projected timetable for the completion of AUV/ biosensor integration is August 2004, and the biosensor will be deployed on AUV or stationary buoys and AUVs subsequent to August 2004, pending acquisition of REMUS AUV. Partners are Tulane and Xavier Universities; COTS Technology, LLC; Sapidyne Instruments (ID); Woods Hole Oceanographic Institute (MA); and the US Naval Oceanographic Office (MS). Patents will be applied for by the partners.

APPENDIX D.

INTELLECTUAL DEVELOPMENT

Intellectual Development

Student Name	Level	Institution	Mentor
Frigo, Dan	Graduate	Tulane University	John McLachlan Ph.D.
Simpson, Erica	Graduate	Tulane University	John McLachlan Ph.D.
Vigh, Katinka	Undergraduate	Tulane University	Matthew Burow, Ph.D.
Johnson, Renee	Undergraduate	Xavier University	Robert Blake, Ph.D.
Borders, Wayne	Undergraduate	Xavier University	Robert Blake, Ph.D.
Bolden, Tiffany	Undergraduate	Xavier University	Robert Blake, Ph.D.
Jackson, Teresa	Undergraduate	Xavier University	Robert Blake, Ph.D.
Harris, Kelley	Undergraduate	Xavier University	Shubha Kale Ireland, Ph.D.
Blumfield, Kristi	Undergraduate	Xavier University	Shubha Kale Ireland, Ph.D.
Kwang, Sarah	Undergraduate	Xavier University	Shubha Kale Ireland, Ph.D.
Williams, Candice	Undergraduate	Xavier University	Tanya McKinney, Ph.D.
Webb, Cristal	Undergraduate	Xavier University	Tanya McKinney, Ph.D.
Njoku, Victor	Undergraduate	Xavier University	Tanya McKinney, Ph.D.
Bolden, Gevoni	Undergraduate	Xavier University	Michael Adams, Ph.D.
Ray, Kamilah	Undergraduate	Xavier University	Michael Adams, Ph.D.
Adewumi, Dare'	SPRITE	Tulane University	Aline Scandurro, Ph.D.
Curtis, Angie	SPRITE	Tulane University	Ken Muneoka, Ph.D.
Fruga, Renada	SPRITE	Tulane University	Gilbert Morris, Ph.D.
Gates, L'Issa	SPRITE	Tulane University	Mansour Mohamadzadeh, Ph.D.
Humbles, Erin	SPRITE	Tulane University	Paul Brindley, Ph.D.
Jones, Maiysha	SPRITE	Tulane University	Glen Boyd, Ph.D.

APPENDIX E.

HISTORICAL DOCUMENTS

- BAA
- Award/Modification Letter(s)
- Timeline
- SF 298 Cover Sheet

ONR 2002

Contracts & Grants

As published in the Commerce Business Daily: 28 Aug 2001

Solicitation Number: BAA 02-001
Due Date: Proposals may be received at anytime during the period from 22 August 2001 through one year thereafter.
Classification: A
Type: Procurement

Agency:
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5660

Title: Long-Range Scientific and Technology Program (2002)

Synopsis:

The Office of Naval Research (ONR) is interested in receiving proposals for Long-Range Science and Technology (S & T) Projects which offer potential for advancement and improvement of Navy and Marine Corps operations. Readers should note that this is an announcement to declare ONR's broad role in competitive funding of meritorious research across a spectrum of science and engineering disciplines. This publication constitutes a Broad Agency Announcement (BAA) as contemplated in FAR 6.102(d)(2). No request for proposal (RFP), solicitation or other announcement of this opportunity will be made. This announcement will remain open until replaced by a successor BAA for approximately one year from the date of publication. Proposals may be submitted any time during this period. Awards may take the form of contracts, grants, cooperative agreements, or other transaction agreements, as appropriate.

Proposal submission is not restricted in any way to any particular entity. All interested responsible sources may submit a proposal. Historically Black Colleges and Universities, Minority Institutions (including Hispanic Serving Institutions and Tribal Colleges and Universities), as well as Small Businesses, HUBZone Small Businesses, Small Disadvantaged Businesses, Veteran-Owned Small Businesses (including Service-Disabled Veteran-Owned Small Businesses), and Women-Owned Small Businesses are encouraged to participate.

Proposal Preparation:

Before preparing proposals, potential offerors are strongly encouraged to contact the ONR Program Officer whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page accessible through World Wide Web at http://www.onr.navy.mil/sci_tech/. THE ONR LONG RANGE BAA 02-001 IS A COMPETITIVE SOLICITATION. PROPOSALS SUBMITTED IN RESPONSE TO THIS SOLICITATION MUST CITE BAA 02-001 ON THE PROPOSAL COVER PAGE. Proposals should also be accompanied by a completed certification package which can be accessed on the ONR Home Page at Contract & Grants, "How to Submit a Proposal. " For grant proposals and proposals for cooperative agreements or other transaction agreements (other than for prototypes), the certification package is entitled, "Certifications for Grants and Agreements. " For contract proposals and for other transaction proposals involving prototypes (Section 845 agreements), the certification package is entitled,

"Representations and Certifications for Contracts. "

For grant awards, it is anticipated that the selected proposal will be incorporated by reference into the grant award. For contract and agreement awards, it is anticipated that the proposed statement of work (SOW) will be incorporated as an attachment to the resultant award instrument. To this end, such proposals must include a severable, self-standing SOW without any proprietary restrictions, which can be attached to the contract or agreement award.

Successful offerors not already registered in the Central Contractor Registry (CCR) will be required to register in CCR prior to award of any grant, contract, cooperative agreement, or other transaction agreement. Information on CCR registration is available at http://www.onr.navy.mil/02/ccr.htm.

All proposals must be submitted in hard copy. It is requested that offerors also provide an electronic copy of their proposals on a 3.5 " Diskette, Zip Diskette or CD-ROM, (in Microsoft & reg; Word 97 compatible or .PDF format). Proposals may not be submitted by fax or e-mail transmission.

If it is anticipated that access to classified material will be required during contract performance, the offeror is requested to clearly identify such need in the technical proposal.

Where to Submit Proposals

Proposals should be addressed to the attention of the ONR Program Officer whose program best matches the offeror's field of interest as follows:

Office of Naval Research
Attn:
Program Officer:_____, Code:_____
800 North Quincy Street
Arlington, VA 22217-5660

Pre-proposals or "White Papers " are frequently desired by ONR Program Officers. Offerors should consult the cognizant ONR Program Officer regarding the desirability of "White Paper " submissions.

Points of Contact

Questions of a technical nature should be submitted to the ONR Program Officer whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page accessible through the World Wide Web at http://www.onr.navy.mil/sci_tech/.

Department of Defense High Performance Computing Program

The DoD High Performance Computing Program (HPCMP) furnishes the DoD S & T and DT & E communities with use-access to very powerful high performance computing systems. Awardees of ONR contracts, grants, and assistance instruments may be eligible to use HPCMP assets in support of their funded activities if ONR Program Officer approval is obtained and if security/screening requirements are favorably completed. Additional information and an application may be found at http://www.hpcmo.hpc.mil/.

Evaluation of Proposals

Award decisions will be based on a competitive selection of proposals resulting from a scientific/technical review. Evaluations will be conducted using the following evaluation criteria: (1) overall scientific and technical merits of the proposal; (2) potential naval relevance and contributions of the effort to the agency's specific mission; (3) the offeror's capabilities, related experience, facilities, techniques or unique combinations of these which are integral factors for achieving the proposal objectives; (4) the qualifications, capabilities and experience of the proposed Principal Investigator, team leader and key personnel who are critical in achieving the proposal objectives; and (5) the realism of the proposed cost and availability of funds. For contract proposals, the socio-economic merits of the proposal will also be an evaluation criterion.

Technical and cost proposals submitted under this BAA will be protected from unauthorized disclosure in accordance with FAR 3.104-5 and 15.207. Government personnel will perform the evaluation of technical and cost proposals. Restrictive notices notwithstanding, one or more support contractors may be utilized as subject-matter-expert technical consultants. Similarly, support contractors may be utilized to evaluate cost proposals. However, proposal selection and award decisions are solely the responsibility of Government personnel. Each support contractor's employee having access to technical and cost proposals submitted in response to this BAA will be required to sign a non-disclosure statement prior to receipt of any proposal submissions.

ONR reserves the right to fund all, some or none of the proposals received under this BAA. ONR provides no funding for direct reimbursement of proposal development costs. Technical and cost proposals (or any other material) submitted in response to this BAA will not be returned.

Use of Animals and Human Subjects in Research

If animals are to be utilized in the research effort proposed, the offeror must complete a DoD Animal Use Protocol with supporting documentation (copies of AAALAC accreditation and/or NIH assurance, IACUC approval, research literature database searches, and the two most recent USDA inspection reports) prior to award. Similarly, for any proposal that involves the experimental use of human subjects, the offeror must obtain approval from the offeror's committee for protection of human subjects (normally referred to as an Institutional Review Board, (IRB)). The offeror must also provide NIH (OHRP/DHHS) documentation of a Federalwide Assurance that covers the proposed human subjects study. If the offeror does not have a Federalwide Assurance, a DoD Single Project Assurance for that work must be completed prior to award. Please see http://www.onr.navy.mil/02/howto.htm for further information.

Industry-Academia Partnering

ONR highly encourages partnering among industry and academia with a view toward speeding the incorporation of new science and technology into fielded systems. Proposals that utilize industry-academic partnering which enhances the development of novel S & T advances will be given favorable consideration.

Socio-Economic Merit Evaluation

For awards made as contracts, the socio-economic merits of each proposal will be evaluated based on the commitment to provide meaningful subcontracting opportunities for Small Businesses, HUBZone Small Businesses, Small Disadvantaged Businesses, Veteran-Owned Small Businesses, Service-Disabled Veteran-Owned Small Businesses, Woman-Owned Small Business Concerns, Historically Black Colleges and Universities, Minority Institutions (including Hispanic Serving Institutions and Tribal Colleges and Universities). In addition, successful contract proposals that exceed $500,000, submitted by all but small business concerns, will be required to submit a Small Business Subcontracting Plan in accordance with FAR 52.219-9, prior to

02/03

AWARD/ MODIFICATION

3a. ISSUED BY:
OFFICE OF NAVAL RESEARCH
BALLSTON CENTRE TOWER ONE
800 NORTH QUINCY STREET
ARLINGTON VA 22217-5660

1. INSTRUMENT TYPE: Grant
3b. CFDA: 12.300
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3c. DUNS NUMBER:

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8. ACTIVITY/AGENCY PROPOSAL NO.:	9. RECIPIENT PROPOSAL NO.:	10. PROPOSAL DATE:	11. ACTIVITY TYPE:	12. PROGRAM TYPE:
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13. ISSUED TO
13a. ADDRESS:
13b. CAGE: 4B966
13c. EDI/EFT NUMBER: 2142AL

TULANE UNIVERSITY
OFFICE OF RESEARCH AND PROJECT
ADMINISTRATION
327 GIBSON HALL
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15. RESEARCH TITLE AND/OR DESCRIPTION OF PROJECT AND/OR PROPOSAL TITLE:
Integrated Bioenvironmental Hazards Research Program

16. FUNDING	ACTIVITY/AGENCY SHARE	RECIPIENT SHARE	TOTAL
PREVIOUSLY OBLIGATED:	$7,522,000.00	$.00	$7,522,000.00
OBLIGATED BY THIS ACTION:	$953,500.00	$.00	$953,500.00
TOTAL OBLIGATED ON AWARD:	$8,475,500.00	$.00	$8,475,500.00
FUTURE FUNDING:	$.00	$.00	$.00
GRANT TOTAL:	$8,475,500.00	$.00	$8,475,500.00

17. CURRENT FUNDING PERIOD
N/A
THROUGH
N/A

18. PERIOD OF PERFORMANCE
01-MAY-1999
THROUGH
31-JUL-2003

19. ACCOUNTING AND APPROPRIATION DATA:
See attached Financial Accounting Data Sheet(s)

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26b. CODE: N00014
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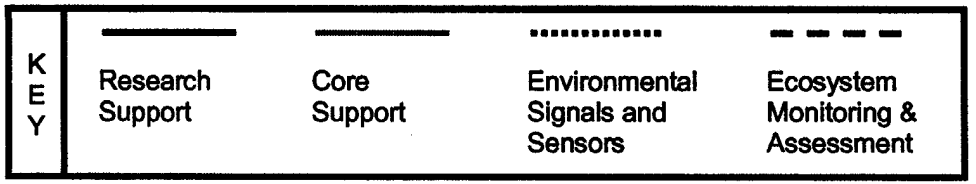
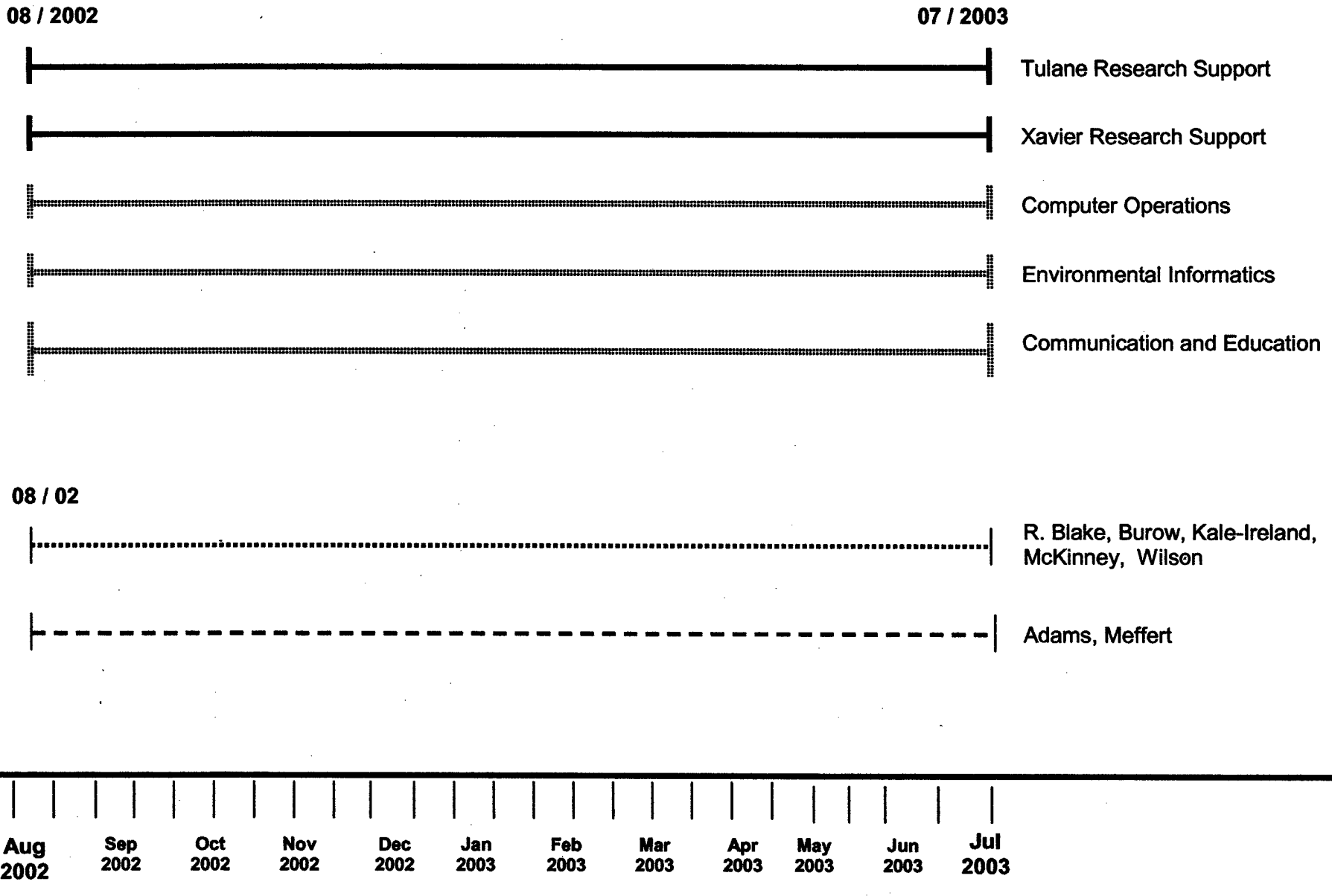
ONR 2002 - 2003 Timeline





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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Since April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research (ONR) to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and make connections between these impacts. One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals at molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health. A second module, Ecosystem Monitoring, emphasizes research on small scale turbulence and the development of biosensors and autonomous platforms for assessments of toxicity and risk. The BHRP program includes mechanisms for effective communication of this information for resolution of DOD problems and for educational training of future scientists. Transcending traditional structures, the CBR has become a model of academic/government/industry interaction.

15. SUBJECT TERMS

Autonomous Underwater Vehicles (AUV), Biosensors, Communication & Education, Environmental Signaling

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					19b. TELEPHONE NUMBER *(Include area code)* (504) 585-6910